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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 48/00, C12N 5/00, 5/06, 5/08, 5/10, 5/12, 5/16, 5/22

A1

(11) International Publication Number: **WO 95/29704**

(43) International Publication Date: 9 November 1995 (09.11.95)

(21) International Application Number: PCT/US95/05330

(22) International Filing Date: 27 April 1995 (27.04.95)

(30) Priority Data:
08/234,429 28 April 1994 (28.04.94) US

(71)(72) Applicant and Inventor: FREEMAN, Scott [US/US]; Apartment No. 309, 333 Julia Street, New Orleans, LA 70130 (US).

(74) Agents: HARE, Christopher, A. et al.; Fliesler, Dubb, Meyer and Lovejoy, Suite 400, Four Embarcadero Center, San Francisco, CA 94111-4156 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: CELL LINES OBTAINED BY *IN VIVO* MIGRATION AND BY FUSION WITH AUTOIMMUNE CELLS

(57) Abstract

The present invention is related to compositions comprising cells, cell lines, or cell populations having properties that allow the cells when injected into a host to migrate to specific tissues to produce an effect against a disease, such as an antitumor effect. Methods of preparation of such cells, and methods of therapy using such cells, both *in vitro* and *in vivo* are also disclosed.

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CELL LINES OBTAINED BY IN VIVO MIGRATION AND BY FUSION WITH AUTOIMMUNE CELLS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is related to compositions comprising cells, cell lines, or cell populations having properties that allow the cells when injected into a host to migrate to specific tissue or cell-types to produce an effect against a disease, such as an antitumor effect. Methods of preparation or selection of such cells, and methods of therapy using such cells, both *in vitro* and *in vivo* are also disclosed.

2. Background of the Technology

In the art of targeting particular tissues or cell types, the typical approach has been to develop antibodies to an antigen that is present in the cell or tissue-type. In diagnostic and therapeutic applications, it is becoming increasingly routine to attach moieties to antibodies which target specific cell or tissue-types and administer them to patients for their diagnostic or therapeutic effect.

However, in the area of "gene therapy" where it is desired to insert a gene into a particular cell or tissue-type, antibodies are not effective. Instead, the gene therapy art has turned with some success to other delivery systems, such as liposomes, DNA conjugants, topical application, direct injection, and the like. Yet, these methods are not always practical strategies in the treatment of certain diseases, such as cancer.

Other researchers have turned to the development of packaging cell lines to house a vector that can be expressed *in vivo*. Typically, such cell lines are

Therefore, it would be advantageous to have a more efficient and specific targeting system. Work by Rosenberg *New Engl. J.Med* **316**:889 (1987) has demonstrated that tumor infiltrating lymphocytes (TIL) can be targeted to specific tissues through the systemic circulatory system. Such cells offer an opportunity for efficiency and specificity that would be advantageous in gene therapy.

It will be noted, however, that TIL are normal circulatory system cells. Such cells are "designed" to pass through the circulatory system. The ability of other cell types to pass through the circulatory system is not clear. Such cells would have to be small enough to pass through the lung. Moreover, such cells cannot contain adhesion molecules or other moieties that would cause them to be processed by the lung or liver or the reticuloendothelial system, in general. Finally, such cells would have to have an appropriate size and deformability to be able to pass into the end organ or tissue and be incorporated therein.

With these constraints, it is not conventional to conceive of cells that possess these characteristics that are not normal circulatory cells. Although, it should be noted that metastatic tumor cells appear to meet these criteria and pass through the circulatory system. However, it is expected that metastatic cells have one or more factors, such as adhesion molecules, that assist them in travelling. See for example, Miyazaki et al. *Cell Biology* **90**: 11767-11771 (1993).

Nevertheless, it would be advantageous to have cells, cell lines, and/or cell populations that will migrate from a site of introduction, to a specific target site in a subject. Such cells would enable the necessary specificity and efficiency that is lacking in many current gene therapy delivery protocols.

SUMMARY OF THE INVENTION

The present invention provides cells, cell populations, and/or cell lines that can migrate to a selected cell or tissue type, such as a tumor, *in vivo* and

methods for identifying and selecting for the same. We have surprisingly discovered that such cell lines can be generated without any manipulation or selection means, provided that the particular cell is adapted to migrate to a specific tissue or cell type *in vivo* or *in vitro*. However, such cells are preferably selected for their ability to migrate, so as to enhance their migration ability.

Cells that will migrate to particular cell or tissue types are referred to herein as migration cells. Such a cells, cell populations and/or cell lines upon further selection, as mentioned above, are referred to herein as migration selected cells (MSC). Together, migration selected cells and migration cells are referred to herein as migration cells (MC). Appropriate migratory cells (MC) can be identified *in vitro* or *in vivo*. For example, for *in vitro* identification, cells that adhere to the specific tissue or cell type will display migratory properties. Alternatively, *in vivo* identification comprises selecting cells that migrate from a site of implantation, or first site, to a second site containing target cells. For example, tumor cells that are physically removed from the first site can act as target cells for tumor cells at a second site in a patient.

The present invention also provides cells, cell lines or cell populations that are generated by fusion of cells associated with an immune response in a donor with pathological cells obtained from a subject, referred to herein as FC. The pathological cells can be associated with a disease or condition in the subject. For example, the pathological cells can be associated with cancer, a virus infection, or an autoimmune disease. Such cells may be obtained from the subject. Methods are described for using the fused cell compositions for treating or killing diseases or conditions *in vivo* or *in vitro*. In addition to treatment, the fused cells in accordance with the invention may be used to detect pathological cells associated with a disease, either *in vivo* or *in vitro*.

a first site of introduction to a subject to a specific cell or tissue-type at a

second site in the subject, comprising providing a first cell population that exhibits associational tendencies with the cell or tissue-type at the second site; introducing the first cell population into the subject at a first site; allowing a portion of the first cell population to migrate to and associate with the cell or tissue-type at the second site; and recovering the portion of the first cell population from the second site that migrated thereto from the first site as the migration selected cell population.

Preferably, the associational tendencies are selected from the group consisting of aggregation with the cell or tissue type *in vitro*, migration to the cell or tissue-type *in vitro*, homing to the cell or tissue-type *in vitro*, migration to the cell or tissue-type *in vivo*, and homing to the cell or tissue-type *in vivo*.

Preferably, in the introducing step, the mode of introduction is selected from the group consisting of injection, implantation, and application.

Also, in one embodiment the cell population comprises cancer cells and the cell or tissue type at the second site comprises cancerous cells. Preferably, the cancer cells are genetically altered to include a gene selected from the group consisting of a susceptibility enhancing gene, a cytokine gene, a lymphokine gene, a histocompatibility antigen gene, a T cell receptor gene, a chimeric cytokine gene, an adhesion molecule gene, a tumor antigen gene, and a virus protein gene. In a preferred embodiment, the gene is a human or murine gene that is selected from the group consisting of a susceptibility enhancing gene, a cytokine gene, and a lymphokine gene. In another preferred embodiment, the cancer cell is selected from the group consisting of ovarian carcinomas, breast carcinomas, leptomeningeal carcinomatosis, colon carcinoma, glioblastoma, a B cell lymphoma, a T cell lymphoma, a B cell leukemia, a T cell leukemia, neuroblastoma, soft tissue sarcoma, cervical carcinoma, lung carcinoma, urinary carcinoma, bladder carcinoma, stomach carcinoma, adrenal cortex carcinoma, endometrial carcinoma, prostate carcinoma, fibrosarcoma, adenocarcinoma, prostate carcinoma, liver carcinoma, pancreatic carcinoma, and biliary duct carcinoma.

In accordance with another aspect of the present invention, there is provided a method to deliver a transduced tumor cell to a tumor in a subject, comprising providing a transduced tumor cell that, prior to transduction, is similar in type to the tumor in the subject, and, wherein upon transduction, the transduced tumor cell comprises a gene that is not present in significant quantities in the tumor, introducing the transduced tumor cell into the subject; and allowing the transduced tumor cell to migrate to the tumor.

In a preferred embodiment, the tumor cell is transduced with a gene that renders it more susceptible to a therapeutic agent. In another preferred embodiment, the gene is a thymidine kinase gene and therapeutic agent is a nucleoside analogue. In another preferred embodiment, the thymidine kinase gene is derived from a herpes virus. In another preferred embodiment, the nucleoside analogue is selected from the group selected from acyclovir and ganciclovir.

In accordance with another aspect of the present invention, there is provided a migration selected cell line, comprising a cell population derived from cells removed from a first cell or tissue type in a subject following migration of the cells from a second site in the subject. Preferably, the cell population comprises cancer cells and the second site of the subject comprises a tumor.

In accordance with another aspect of the present invention, there is provided a method for treating cancer in a first subject having a site of cancerous tissue, comprising removing a population of cancerous cells from the first subject; transducing the cancer cells with a gene that renders the cells more susceptible to a therapeutic agent; introducing the transduced cancer cells into the first subject and allowing the transduced cells to migrate to the site of cancerous tissue; and treating the subject with the therapeutic agent, wherein, upon death of the transduced [illegible]

In a preferred embodiment, the gene is a thymidine kinase gene and therapeutic agent is a nucleoside analogue. In another preferred embodiment, the thymidine kinase gene is derived from a herpes virus. In still another preferred embodiment, the nucleoside analogue is selected from the group selected from acyclovir and ganciclovir.

The method may additionally comprise, prior to the introducing step, the steps of: introducing the transduced cancer cells into a second subject at a first site, the second subject having cencerous cells at a second site of a similar type to the cancerous cells in the first subject; allowing the transduced cancer cells to migrate to the second site in the second subject; and removing the transduced cancer cells from the second site of the second subject.

The method may additionally alternatively comprise, prior to the transducing step, the steps of: introducing the transduced cancer cells into a second subject at a first site, the second subject having cencerous cells at a second site of a similar type to the cancerous cells in the first subject; allowing the transduced cancer cells to migrate to the second site in the second subject; and removing the transduced cancer cells from the second site of the second subject.

In either of these embodiments, the second subject may be allogenic or xenogenic with the first subject or the second subject may be genetically similar to the first subject. For example, the second subject is a mouse and the first subject is a human.

In accordance with another aspect of the present invention, there is provided a method to induce cytokine production in a subject having a tumor, comprising: providing a population of modified cancer cells that are similar in type to cells in the tumor in the subject, the cancer cells including a gene that renders the cells more susceptible to a therapeutic agent; introducing the modified cancer cells into the subject in a manner designed to bring the cancer cells into proximity with the tumor; and administering the agent to the subject

in order to kill the modified cancer cells, wherein, upon the death of the modified cancer cells, cytokine production is induced in the tumor.

In a preferred embodiment, the gene is a thymidine kinase gene and therapeutic agent is a nucleoside analogue. In another preferred embodiment, the thymidine kinase gene is derived from a herpes virus. The nucleoside analogue is preferably selected from the group selected from acyclovir and ganciclovir. In accordance with the method, the cytokine production that is induced is selected from the group consisting of IL-1α, IL-6, TNF-α, IFN-γ, and GMCSF.

In accordance with another aspect of the present invention, there is provided a fused cell cell line, comprising: a cell line derived from T helper 2 cells fused with a tumor cell line from a host animal.

In accordance with another aspect of the present invention, there is provided a fused cell cell line, comprising: a cell line derived from CTCL cells fused with a tumor cell line from a host animal.

DETAILED DESCRIPTION OF THE INV. TION

The present invention is directed to the use of cells as carriers to target or direct therapies to selected tissue types. The mode of action or operation, by way of example and without limitation, can include the delivery of therapeutic agents or effect or the creation of an immune response. In accordance with the invention, therefore, there are provided methods to identify, obtain, and select cells for the above-purposes. Also provided are methods of therapy of diseases utilizing such cells, such as the treatment of cancer.

In general, the present invention focuses on the use of the migration of cells to target a therapy and on the ability of certain cells to elicit an immune response in a patient. Cells which will [illegible]

[illegible] invention to migrate faster and or more efficiently. Selected migration cells are

referred to herein as migration selected cells (MSC). In another embodiment a fusion cell, an (FC), is used to elicit an immune response in a subject. In a preferred embodiment, FC are prepared from fusing a cell associated with an immune/inflammatory cell from a donor with pathological cells obtained from a subject. The pathological cells can be associated with a disease or condition in the subject. For example, the pathological cells can be associated with cancer, a virus infection, or an autoimmune disease.

I. ***Application of the Invention to "Bystander Effect" Cancer Therapy***

The invention is especially well suited to the treatment of cancer. In WO 93/02556 (incorporated herein by reference in its entirety), "bystander effect" cancer therapy was disclosed. In such therapy, when a tumor cell, transfected with a gene that renders the cell more susceptible to a therapeutic agent, is placed in proximity to cancerous tissue or cells in a subject, upon treatment of the subject with the therapeutic agent to which the transfected cells are susceptible, regression of the cancerous condition in the subject is observed. For example, a tumor cell line transfected with the thymidine kinase gene from herpes simplex virus ("HSV-TK" gene) when placed in proximity to a tumor in a subject will cause tumor regression in the subject upon nucleoside analogue treatment, such as ganciclovir or acyclovir.

The specific mode of action for this effect is unclear. However, it appears to involve both an immune component as well as a drug susceptibility component. Transfected cell proximity to the tumor appears to assist the process. In the patent application (WO 93/02556), it was suggested that homing of transfected cells could occur, i.e., where the transfected cancer cells would preferentially aggregate around the tumor to facilitate proximity of the cells to the tumor or cell-to-cell contact.

We have now surprisingly discovered that homing can indeed be used to direct transfected cancer cells to the site of a tumor. For example, tumor cells transfected with the HSV-TK gene can be injected into a subject having

one or more tumors, at a site remote from the tumor, and the cells will migrate to the tumor. Subsequent treatment of the subject with ganciclovir, for example, will cause tumor regression in the subject.

As described in WO 93/02556, tumor cells that carry and express an inserted HSV-TK gene ("TK+ tumor cells") are potent therapeutic agents for treatment of tumors or malignant conditions. Tumor cells that do not express or that do not contain an inserted herpes virus TK gene ("TK- tumor cells") will have only (or mostly) thymidine kinase which is expressed by endogenous tumor cell thymidine kinase genes. TK- tumor cells only have cellular thymidine kinase which is a thousand fold less effective in the phosphorylation of ganciclovir/acyclovir into its active form than TK+ tumor cells. Hence a useful therapeutic index is associated with the treatment.

The mechanism that underlies the therapeutic efficacy of such cells is not fully understood, but is based at least in part on killing of the TK+ tumor cells in a subject when an appropriate nucleoside analog, such as ganciclovir, is administered to the subject, i.e., is associated with the presence of the inserted TK gene. The TK+ tumor cells are killed by an incorporation of ganciclovir triphosphate into cellular DNA by DNA ploymerases in the TK+ tumor cells. Ganciclovir triphosphate synthesis is facilitated by the inserted TK gene which appears to catalyze or facilitate phosphorylation of ganciclovir through the synthesis of the mono-, di-, and triphosphate.

However, immune mechanisms also appear to be invoked in TK+ tumor cell killing *in vivo*. Killing of tumor cells in a subject that do not carry the TK gene also occurs, and this is believed to be mediated by more than one mechanism, such as the following mechanisms and any combination thereof. One possible explanation is the direct transfer of ganciclovir and ganciclovir mono-, di-, and/or triphosphate from TK+ tumor cells to nearby TK- tumor cells. Another possibility is the transfer of [illegible] other cells [illegible] nearby TK- tumor cells. Alternatively, cytotoxic immune

responses could presumably be directed to tumor antigens (primarily cell surface antigens) which leads to killing of TK- tumor cells (where such TK- tumor cells are not necessarily near the TK+ tumor cells). Also, humoral immune responses directed to tumor antigens which leads to killing of TK- tumor cells (where such TK-tumor cells are not necessarily near the TK+ tumor cells) could be implicated. In addition, we have now demonstrated that cytokines and/or other soluble factors are released in the cancerous tissue upon the therapy. For example, we have now demonstrated that intraperitoneal injection of TK+ cells into mice having an intraperitoneal tumor and treatment with ganciclovir leads to production of interleukin-1 (IL-1), interleukin-6 (IL-6), granulocyte macrophage stimulating factor (GMCSF), and interleukin-alpha (IFN-α). In addition, the presence of tumor necrosis factor (TNF) has been detected by immunohistological staining of tumor cells.

The fact that TK- tumor cell killing is mediated, at least in part, by immune responses demonstrates that TK+ tumor cells can be useful as a tumor vaccine in human and nonhuman subjects including mice and rats.

In the context of the present invention, migration cells derived from tumor donor cells and carrying and expressing an inserted TK gene ("TK+ tumor MSC") are useful for treating tumors or malignant conditions in human and nonhuman subjects. TK+ tumor MSC derived from tumor cells are used in the same manner as described for the use of TK+ tumor cells (WO 93/02556). Killing of TK+ cells appears to occur by a mechanism of necrosis or apoptosis. Necrosis is characterized by swelling of the cell, disintegration of the cell membrane and nuclear flocculation. Apoptosis is characterized by cell shrinkage, membrane vesicle formation and condensation of chromatin (Cotter *Anticancer Research* **10**:1153 (1990)). Killing of parental cells in the presence of ganciclovir or another suitable nucleoside analog may be due in part to death of TK+ cells by apoptosis which results in tumor "immunity" in a subject.

II. *Other Therapeutic Procedures*

As will be appreciated, the invention is useful for more than the treatment of cancer through the bystander effect described above. For example, the invention has broad utility in other cancer therapies, such as delivery of cytokines through the transfection of cancer cells with a cytokine gene. In addition, the invention can also be used to treat a variety of other disease states or tissues. Essentially, all that is required is to select a cell type that will migrate to the particular tissue which is diseased or cell-type that is responsible for the disease state in the subject (i.e., a migration cell (MC)). The MC is generally manipulated to, for example, render it more susceptible to therapy, secrete a therapeutic agent, and/or cause an immune response in the subject.

This step of manipulation may be considered "activation" of the cell to allow or enhance the therapeutic effect. It will be understood that, in certain therapeutic instances, activation is not necessary. For example, where the MC is used to stimulate an immune response. Even here, however, some manipulation of the cell may be desired to promote or enhance the therapeutic action of the cell or the immunogenicity of the cell. For example, the MC can be manipulated to include a colony stimulating factor or an antigen. Appropriate colony stimulating factors include granulocyte-macrophage colony stimulating factor (GMCSF), granulocyte colony stimulating factor (GCSF), monocyte colony stimulating factor (MCSF), and others. An appropriate antigen is dinitrophenol (DNP), for example. Indeed, in a majority of situations, some manipulation of the cell is necessary to achieve the desired therapeutic effect.

We have also discovered that it is possible to create fusion cells that will elicit an immune response in a subject, herein FC. The FC can be either MC (i.e., migrate within the subject) or can merely be immunogenic. Preferably,

pathological cells can be associated with a disease or condition in the subject.

For example, the pathological cells can be associated with cancer, a virus infection, or an autoimmune disease. Such cells may be obtained from the subject or from another host.

Intriguingly, the FC of the present invention can be used therapeutically in a similar manner as the MC of the present invention. Similar to MC, the operation of FC may be "remote," i.e., no cell-cell proximity is necessary for their therapeutic effect. Moreover, the FC of the present invention can additionally incorporate other moieties, such as genes or agents that will cause an enhanced effect.

The migration cells (MC) of the invention are obtained by identifying cells that can preferentially target a cell or tissue type in a subject. Fusion cells (FC) of the invention, may also act as MC. Alternatively, FC may deliver a toxic molecule or stimulate an immune response in a tumor or other disease state, while acting as an MC or not. Both MC and FC can act to immunize a subject or the host to a particular disease upon injection or implantation into the subject or host. MC and FC can also confer responsiveness to certain therapies. For example, such cells can be transfected with genes which render them susceptible to certain therapies, transfected with a gene that causes them to secrete therapeutic agents, or include an agent that renders them more susceptible to electromagnetic radiation, such as phototherapy.

In addition to the use of MC or FC of the present invention in classical therapy, the cell lines or populations may also be used for a variety of other exemplary approaches. For example, such cells can be used to eliminate or purge cell populations *in vitro*, such as the purging of tumor cells from bone marrow cells in tissue culture. Also, the cells can be used to detect or locate tumor cell masses *in vivo* (i.e., through the use of markers, such as radionuclides) or *in vitro* (i.e., through detecting aggregation or through markers). In addition, the cells can be used to detect antigens in a mixed lymphocyte assay.

A variation of the invention can use MC containing only GMCSF, TK, or another cytokine as an aid in the treatment of a tumor or malignant condition. In other aspects of this embodiment, TK and/or a cytokine such as IL- 1, IL-2 or GMCSF is inserted into the MC along with an expressed tumor antigen (cell surface protein) gene, a fragment of such a gene or a histocompatibility allele that will elicit an immune response in the subject against the cell surface protein.

II. *Combination Therapies*

The present invention includes the use of the MC or FC in combination with conventional therapies. For example, when MSC are used to treat a cancer or tumor in a human or nonhuman subject (mouse, rat, rabbit, dog, etc), such treatment can be combined with other conventional therapies including radiation treatments, chemotherapy or surgical resection of tumor masses.

Virus infections that are treated using MC can also be combined with antiviral agents such as 3'-azidothymidine, (-)-2'-deoxy-3'-thiacytidine, (+)-2'-deoxy-3'-thiacytidine, 2',3'-dideoxycytidine or 5 2',3'-dideoxyuracil for HTLV-1, HIV or HBV treatment in humans or murine leukemia virus treatment in the mouse, human HIV in the SCID mouse or feline leukemia virus in the cat, ganciclovir for human CMV or murine CMV treatment or acyclovir for human HSV-1 or HSV-2 treatment.

MC can be used in protocols to screen for new therapeutic agents, such as antivirals or anticancer agents, that are more effective in subjects treated with such cells (to stimulate host immune responses or other responses that can lead to or facilitate killing of pathological cells *in vivo*). In these cases, MC are useful as a tumor vaccine or as a virus vaccine for stand alone treatments or MSC can be used in combination with other known therapies.

[illegible] MC [illegible] can be used

embodiment, a surgeon surgically removing a tumor saves the tumor cells from

the patient for *in vitro* culture. Once the culture is established, MC can be identified and selected, as described above, through either *in vivo* or *in vitro* methods. The cells can be transduced with a vector, i.e., the TK gene and/or a cytokine gene (i.e., IL-1) and preferably a marker (i.e., the neo gene), either before or after selection. It is preferred that the cells be transduced with some form of a marker before selection so that they can be recognized.

Advantageously, for example in the case of a human tumor, a surgeon can remove the tumor, raise the cells in culture, and perform selection methods *in vivo* in an animal other than a human. For instance, genetically identical mice with tumors can be used, successively, as a tumor target for the human MC tumor cells. After the MC have had an opportunity to migrate to the tumor, the mouse tumor can be excised and the human tumor cells that migrated can be selected. For example, selection can be accomplished by G418 or neomycin resistance, if such markers were included in the MC. Alternatively, if such markers were not included, cell sorting or genetic marking can be pursued.

The human tumor cells, so selected, will be MSC. The MSC derived from the process (i.e., IL-1) can be introduced into the human subject. Such MSC will target any remaining tumor cells, either in the original site or in a metastatic site. Then, therapy can be commenced. In the case of a TK transfected MSC, treatment with ganciclovir will be used to kill any remaining tumor cells at the original site or at metastatic sites.

III. *Migration cells (MC)*

As used herein, a migration cell (MC) (sometimes referred to herein as a donor cell) means any cell that will migrate toward a selected tissue or cell type. MC can be normal or pathologic cells isolated from the host (autologous), another host (allogenic or xenogenic), or an established cell line (usually allogenic or xenogenic). In some cases, MC are introduced into the subject at a first site and will migrate to a second site. As mentioned, while the

MC can be a normal cell, it is usually a pathological cell, i.e., a cell or cell line associated with a disease or an unwanted physiological condition, including cancer cells, virus infected cells, cells associated with an autoimmune response or disease, cells associated with rejection of an organ transplanted into the subject, and the like.

Such cells can be further selected for their migration qualities. Cells that are further selected for their migration qualities are sometimes referred to herein as migration selected cells (MSC). Alternatively, they may be referred to as MC, since MSC are MC with improved or enhanced migration qualities.

Selection of the MSC can be accomplished either *in vivo* or *in vitro*. Typically, selection is accomplished *in vivo* through introducing a cell (usually an MC) into a subject and after the passage of a period of time sufficient for the cell to migrate to the selected tissue at a second site, the MC that has migrated to the second site is referred to as a migration selected cell (an MSC). An MSC that has migrated to a second site *in vivo* can be recovered from the subject, by means such as biopsy, leukapheresis and the like, and expanded in tissue culture to obtain an MSC population or cell line.

One method of *in vivo* selection includes selection in a subject that is not the host/donor of the cells. In this embodiment, human donor cells can be selected for MSC by using an immunosuppressed or immuno-compromised (i.e., through irradiation or drug treatment, such as cyclosporin A) animal having an appropriate cell or tissue-type to target to with the human donor cell. In addition to immunosuppressed subjects, multiple subjects can be used sequentially (i.e., genetically identical mice used successively for targeting). In this latter embodiment, it will be appreciated that the level of immune response to the donor cell in the allogenic or xenogenic target host should be relatively low. If, however, the same target host were re-used, it is expected

In the above process of *in vivo* selection, the donor cell or MC can be introduced into or to the subject by any suitable means such as by injection into a vein, artery, muscle, organ tissue such as liver, thymus, intestine or CNS tissue or by implantation of donor cells on a solid support such as a suitable collagen matrix at any such site.

In addition to *in vivo* selection, MSC can also be generated using *in vitro* tissue or organ culture methods or by a combination of *in vitro* and *in vivo* methods. Another method to isolate MSC is by identifying donor cells or MSC that adhere *in vitro* to other cell types including tumor cells or other target cells. Another method to isolate MSC is to identify a cell population that naturally homes to other cells *in vitro* or *in vivo*.

In *in vitro* identification of MC, one method is a confluent plate selection. In this method a particular tumor cell that is desired to be targeted is grown to confluence on a plate so that there is no room for additional cells to bind to the plate. Such cells will adhere to the plate. The proposed MC with suitable markers, such as the G418 resistance marker (i.e., the neo gene), are applied to the plate and the plate is washed to remove non-adhering cells. Thereafter, the plate is washed to remove all of the cells and the cells are selected for resistance (i.e., in the case of G418 resistance markers, G418 or neomycin resistance). Resistant cells remaining will be MSC. The process can be repeated to enhance the migration potential of the MSC.

A similar procedure can be carried out on a column or other suitable support. Essentially, all that is required is a way to separate cells that exhibit associational tendencies with the desired target cell or tissue-type and select those cells.

For example, selection can also be accomplished through flow cytometry, measuring size or densities. As will be appreciated, non-adhering cells will pass through the flow cytometer as a single cell. Adherent cells will pass through the cytometer as clumps of two or more cells. The difference between single cells and multiple cells will be readily detectable. Alternatively,

fluorescense activated flow cytometry can be used, such as FDG-FACS. FDG-FACS is accomplished as follows: Cells are trypsinized and resuspended in M199 + 5% FCS at a concentration of approximately 10^7/ml. 100 μl of the cell suspension is warmed to 37°C and mixed with 100 μl of prewarmed (37°C) 2 mM FGD in water. The FDG-cell mixture is incubated at 37°C for exactly one minute and 1.8 ml of ice cold M199 + FCS is added. By incubating the FDG-containing cells on ice for one hour a maximum amount fluorescein is released. The cold temperature "freezes" the cell membranes, trapping both FDG and free fluorescein within the cell. Cells are sorted using a chilled water jacket to prevent leakage of fluorescein from the cells. See Nolan et al. *Proc. Natl. Acad. Sci. USA* **85**:2603 (1988).

As mentioned, an MSC population refers to cells that will migrate to a specific tissue or cell type in a subject (herein a target tissue). The target tissue may be diseased or normal tissue. For example, the target tissue may be a tumor deposit, other diseased tissue, and the like. In the *in vivo* selection process described above, preferred MC are generated through recovering cells from the second site that migrated to the second site fr[illegible] a first site in a subject after at least one round of *in vivo* selection. Other preferred MC are obtained after two, three, four, five, or more rounds of *in vivo* migration/selection.

An MSC population may also be characterized by an ability to migrate to the second site faster than an MC prior to selection. Preferably, the enhancement in the rate of migration relative to MC is about 10-50% faster (i.e., 10%, 15%, 20%, 25%, 30%, 35%, etc., faster than MC). Relative speed of MSC versus MC migration can be determined by *in vivo* comparative studies. For example, running tests on a series of animals with several animals receiving MC and other animals receiving MSC and performing biopsies of the

[illegible] example, in preferred embodiments, the enhancement in efficiency is about

10-200% relative to MC (i.e.,10%, 30%, 50%, 75%, etc. more efficient than MC). Efficiency may be gauged by the difference in the number of MSC that are obtained from a given second site compared to the number of MC implanted at a first site. The first site may appropriately be, for example, mouse or rat tail vein or cephalic vein, etc. The second site can appropriately be, for example, the spleen, a tumor, at a site fixed via implantation of tumor cells in a subject, the thymus, the brain cortex, the wall of a vein or an artery at a given location, etc.

In preferred embodiments, the MC or MSC are additionally "activated." Generally, activation is used herein to indicate that the cell has been manipulated to have one or more enhanced properties, such as increased susceptibility to a therapeutic agent or enhanced migration potential. Activation can include genetically-engineering the cell to contain a selectable marker or a resistance gene. Examples of selectable markers or resistance genes include the neomycin resistance gene (neomycin phosphotransferase, hereafter "neo"), the gpt gene (which confers resistance to micophenolic acid), dihydrofolate reductase (DHFR), and others. Alternatively, or in addition, the cell can be genetically-engineered to contain a susceptibility enhancing gene. Examples of susceptibility genes include herpes virus (HSV-1, HSV-2, CMV, etc.) thymidine kinase gene (TK), cytosine deaminase, and others. Also, the cell can be genetically-engineered to contain a cytokine or lymphokine. Examples of cytokines or lymphokines include the variety of interleukins (including interleukin 1 (IL-1) through interleukin 13 (IL-13) (IL-2, IL-3, IL-4, etc.)), granulocyte-macrophage colony stimulating factor (GMCSF), granulocyte colony stimulating factor (GCSF), monocyte colony stimulating factor (MCSF), interferons, tumor neurosis factor (TNF), and others. The cell can also be engineered to contain or express proteins, such as cell adhesion molecules. Cell adhesion molecules generally will assist in adhesion and/or migration. Examples of cell adhesion molecules include selectins (i.e., E-selectin), members of the immunoglobulin superfamily of adhesion molecules (i.e.,

ICAM-1, ICAM-2, N-CAM, ELAM-1, VCAM-1, and others), cadherins (i.e., E-cadherin), integrins, and extracellular matrix (ECM) molecules. Adhesion molecules are reviewed in Hynes et al. *Cell* **68**:303-322 (1992), the disclosure of which is hereby incorporated by reference. The cell can also include another gene type. For example, autologous, allogenic, or xenogenic histocompatibility antigen genes may be present, to name a few.

MC can be autologous, i.e., derived from the subject itself, or the donor cell can be from an allogenic or xenogenic source. Also, MC can include cells from the second site or from other cell types especially including lymphocytes, white blood cells, monocytes, macrophages, myocytes, keratinocytes, endothelial cells, glial cells, and others. Generally, MC are of the same type as cells commonly found at the second site (e.g., donor lymphocyte recovered from a lymph duct). Or, the donor cells may be of a different type compared to cells commonly found at the second site (e.g., donor lymphocyte recovered from brain tissue).

MC or donor cells can also be normal, transformed, or malignant. The donor cell or MC can be treated *ex vivo* prior to introduction into a host. For example, the cells can be treated with photopheresis (e.g., psoralen and UVA irradiation). The cells can also produce viral vectors, such as a helper virus, to, for example, facilitate in the transfer of a gene(s). The cells can be, or from, a homogenous cell line, clone, or can be a mix of cell types.

It is to be understood that when a particular gene or protein is referred to, such as GMCSF, IL-2, IL-3 or IL-4, for example, the human designation for the protein will usually be given, but the corresponding gene or protein from other species is usually meant to be included, unless the same gene or protein in another species has a biological function that would interfere with the intended purpose. Such a corresponding gene or protein can have a different designation.

For example African green monkeys, cynomologous monkeys, chimpanzees,

baboons) and domestic or other animals such as a horse, cow, dog, cat, chicken, pig, emu, ostrich, trout, salmon, bison, deer, mouse, rat, guinea pig, rabbit, and the like. Subject is also intended to include various groups or subsets of species such as the nonhuman species subset (which includes all species given under subject but excludes humans) and other subsets such as rodent, nonrodent, primate, nonprimate, avian, nonavian species or any combination thereof. Whenever the term subject is used in the present specification, any species or subset of the subject species is intended, unless otherwise specified.

In general, sets of species will be indicated with exemplified embodiments, however, such indicated subjects are not inclusive of all subjects or subsets of subjects that are suitable and intended for the indicated embodiment, unless otherwise stated.

As mentioned above, the first site is usually a vein or artery, but also includes the peritoneum, muscle, central or peripheral nervous system (cerebral cortex, hippocampus, medulla, spinal cord, peripheral nerve or ganglion, etc). There can be more than one first site (e.g., two different muscles, two different veins or arteries, a muscle and a vein/artery, a muscle and peritoneal cavity, lymph duct and a muscle and the like) that is used to introduce a given MC. Alternatively, the first site can be an organ such as liver, kidney, spleen, thymus, and the like. The second site refers to target cells, tissues, organs or pathological structures (i.e., tumors, virus infected cells, liver, spleen, brain, intestine, muscle,etc).

As used herein, migration refers to the movement of MC, donor cells, or MSC to a particular cell or tissue type. Such migration can be *in vivo* or *in vitro*, and *in vivo*, can include movement caused by the subject such as movement through the blood stream and movement by the donor cell itself such as a donor cell migrating through a capillary wall. Migration also includes homing or metastasis of donor cells from a first site to a second site. Migration will generally occur over a time period of from about 2 hours to about 14 days

(i.e., about 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, 84, 96, 120 hours, 6, 7, 8, 9, 10, 12, 14 days etc). Migration of MSC can be faster or more efficient where the MSC that have been selected one or more times compared to donor cells. Migration can be used to select for cells in a donor population that have an enhanced capacity to migrate *in vivo* compared to the migration capacity of an "average" donor cell in a population that has not been subject to migration *in vivo*. In addition, other methods such as genetic engineering of donor cells, *in vitro* selection of migration, or identifying cell lines that naturally migrate can be used to isolate MSC.

Several factors are believed to be responsible for migration. These may include cell surface molecules or soluble factors released by the cells. As the specific mechanisms and molecules responsible for migration are elucidated by studying MSC, the genes associated with their homing or migration properties can subsequently be incorporated into a cell line or population to generate MSC. Thus, if cell surface adhesion molecules are found to play a significant role in migration to a specific tissue then the gene(s) can be incorporated into the donor cells prior to infusion to generate a MSC. Further study of the cell lines and populations identified by the methods described herein will permit identification of the molecules that allow tumor cells and other cell types, such as stem cells that home to bone marrow, myoblasts that migrate to muscle tissue, or dendritic cells that migrate to lymphoid tissues, and the like to preferentially home or migrate to such tissues.

The present application describes methods to identify and use tumor cells that have an enhanced capacity to home to tumor deposits or disease sites. After cell lines having enhanced or heightened homing or migration properties are identified, the cell lines can be used to identify the molecules which confer enhanced homing properties or the cells can be used for therapeutic and diagnostic/assay purposes. The cell lines [illegible]

cells and/or to express other desired properties, such as secreting lymphokines or expressing a desired gene.

An aspect of donor cell migration in a subject is that MSC obtained after migration or homing of implanted donor cells can be recovered from a selected second site shortly after implantation, say 2 to 48 hours after implantation, or MSC can be recovered at later times such as 48 hours to 14 days after implantation. The MSC obtained at different times after implantation of donor cells can have different characteristics with regard to, for example, longevity of expression of genes inserted into the donor cells by genetic engineering (e.g., a susceptibility marker such as the TK gene or a resistance marker such as the neo resistance gene) or the relative level of expression of donor cell gene(s) responsible for eliciting a rejection response by the subject (e.g., xenogenic or allogenic cell surface antigens such as known surface proteins including histocompatibility antigens, glycoproteins, carbohydrates and the like). Individual cells in a donor cell/MSC population that express little or no surface antigen responsible for eliciting a host rejection response against the donor cell/MSC will tend to survive longer *in vivo* than "normal" donor cells or MSC that are better targets for immune-mediated destruction by normal mechanisms, such as CTL20 mediated donor cell/MSC killing.

IV. *Homing*

Migration of MC *in vivo* is believed to involve, at least in some cases, homing of a given cell type to a location containing a similar cell or tissue type. For example, tumor cells obtained from a subject, will unexpectedly migrate to sites in the subject containing resident tumor cell masses or tumor cell concentrations. The precise nature of the differences between a donor cell population and a MC derived therefrom are not fully understood. The differences can be due in some cases to an epigenetic mechanism that causes different levels of expression of gene(s) involved in cell adhesion and/or chemotaxis.

Alternatively, MSC are in some cases, enriched for subpopulations in the original donor population that are heterozygous or homozygous for a mutation(s) that affects the cell's ability to home or migrate. In either case, *in vivo* selection for MC populations is useful as a method to obtain such cells by isolation of MC after 1, 2, 3 or more rounds of migration *in vivo* followed by recovery of a MSC population. Individual clones can be obtained from a MSC population after an given round of selection, i.e. after the 1st, 2nd, 3rd, 4th, etc., round of migration in a subject.

The clones are obtained by any convenient method, such as, for example, by growing single cells in tissue culture to obtain cell colonies. Colonies may be obtained through, for example, selection for cells expressing an inserted neo gene in medium containing G418.

V. *Migration Cells From Dendritic Cells*

In a preferred embodiment of the invention, dendritic cells from a human (i.e., as discussed in WO 93/20186, the disclosure of which is incorporated by reference), mouse, rat, monkey or other sources are used to generate MC. Such cells constitute a system to present antigens to the immune system of a subject, which functions to initiate certain immune responses such as rejection of a transplanted organ, sensitizing MHC-restricted T cells and eliciting T cell dependent antibody formation. In one aspect of this embodiment, human donor cells obtained from a source such as umbilical cord blood, lymph ducts, lymph nodes, bone marrow or peripheral blood are enriched for CD34+ hematopoietic stem or progenitor cells and separated from adherent cells and from non-adherent mononuclear cells by, for example, selection by indirect immune panning using commercially available anti-CD34 antibodies (as described in WO 93/20186. In other aspects of this embodiment, [illegible] tumor necrosis factor-α (TNF-α) and interleukin-3 (IL-3) or in the presence of GM-

CSF to give rise to CD 1 a+ dendritic cells which are obtained according to WO 93/20186. The human CD 1 a+ cells are then used to generate MC by injection into a syngenic (human or nonhuman) or allogenic subject followed by recovery of the cells from the subject and expansion in tissue culture. Prior to *in vivo* migration/selection, human or nonhuman dendritic cells can be genetically engineered to contain genes such as the TK gene, the neo gene and/or cytokines including GM-CSF or IL-3 and the like.

Therapeutic applications for MSC derived from human dendritic cells includes their use to generate CD 4 + helper T cells (useful for adoptive immunotherapy and the like) and includes their use to generate cancer-specific or virus-specific CD8+ cytotoxic T cells for in vitro assay/diagnostic use or for use in a subject.

MC obtained from CD 1 a+ cells can be used in mixed lymphocyte reactions by providing and coculturing responder cells (lymphocytes that proliferate and become cytotoxic T cells in the presence of suitable antigen presenting cells, i.e., MC derived from dendritic cells) from a host that has been exposed to an antigen with inactivated MC obtained from dendritic cells (which act as stimulator cells; such MSC are usually allogenic with respect to the responder cells) where the MSC are obtained by one, two or more rounds of *in vivo* migration in a syngenic, allogenic or xenogenic subject. After coculturing responder cells and the MSC stimulator cells, the response of the responder cells, typically cell proliferation (i.e., as measured by ^{3}H-thymidine uptake or counting dividing cells) or measuring cytokine production by the responder cells, is determined. Responder cells, can be syngenic, allogenic or xenogenic relative to the stimulator MSC. Responder cells will typically contain CD4+ T cells from a subject that will be the recipient of a transplant or are from a recipient that is infected with a virus such as a retrovirus (nonhuman retroviruses such as murine leukemia virus, feline leukemia virus or human retroviruses such as HIV, HTLV-1, etc.), a herpes virus (HSV-1 (human herpes simplex virus, type 1), HSV-2 , CMV (human or murine

cytomegalovirus), Epstein Barr virus) or other type of virus (influenza, human hepatitis B virus, measles virus, enteroviruses and the like).

In another embodiment, MC are derived from tumor infiltrating lymphocytes (TIL) which are described in Rosenberg *New Engl J Med* **316**:889 (1987). TIL are believed to mediate cytotoxic immune responses against certain cancers. The TIL are explanted from tumors (such as melanomas, Kaposi sarcomas, fibrosarcomas and the like) in human or nonhuman subjects and grown in tissue culture *in vitro*. Following growth in tissue culture, the cells are transduced with a vector such as a retroviral vector or an expression vector that is transfected into the cells. The TIL are then selected for growth of only those TIL that have and express vector genes. Thereafter, the selected TIL can then reinfused back into the subject. 1 to 3 days following implantation into the subject, MSC cells are removed, grown again in tissue culture and reimplanted into the subject by, for example, injection into a vein.

A number of the genetically-engineered MSC target or return to tumors *in vivo* after implantation into the subject. MSC from TIL cells are preferably obtained after 2, 3, 4 or more rounds of implanting and recovering the genetically engineered cells from the subject.

V. *Cell Lines and Methods of Use*

The use of various cell lines, growth factors and/or methods to transfer genes into such cells and/or methods to implant cells into human and nonhuman subjects has been discussed (see, for example, U.S. Patent Nos. 5,270,172; 5,270,171; 5,270,191; 5,270,082; 55,256,410, 5,238,839, 5,236,905; 5,263,823; WO 93/25216; WO 93/20186; WO 93/19777; WO 93/19660; WO 93/19591; WO 93/19191 ; WO 93/14790; WO 93/14188; WO 93/10218; WO 93/07281; WO 93/04167; WO 93/03768; WO 91/02805; WO 93/01281; EP

England) 51:[illegible] (1991); *Proc Nat'l Acad Sci U.S.A.* 90:7024 (1993); *Proc*

Nat'l Acad Sci U.S.A. **89**:182 (1992); *J Immunol* **151**:1663 (1993); Paal-Henning, *Cancer Res* **53**:5158 (1993) (each reference is incorporated herein in its entirety by reference).

Useful cell lines or populations and methods to use them may be found in: Freeman *Cancer Res* **53**:5274 (1993), Moolten *J Natl Cancer Inst* **82**:297 (1990) and Moolten *Cancer Res* **46**:5276 (1986). The use of tumor or malignant cells as recipients for an inserted gene that provides a therapeutic target for cancer therapy is described in WO 93/02556.

VI. *Fusion Cells*

Fusion cells (FC) are the product of two or more cell types fused together such that the FC retain certain characteristic biological properties (such as helper capacity for fused T-helper, Th, cells) of the two different cell types. In a preferred embodiment of the present invention, FC are derived from cutaneous T cell lymphoma (CTCL) cells. CTCL is a disease responsive to phototherapy which results in tumor regression mediated at least in part by an anti-tumor immune response.

A vaccine for non-CTCL tumors can be prepared in accordance with the invention through fusing (a) Th cells (Thl or Th2 cells) that mediate CTCL formation and (b) non-CTCL tumor cells from another type of tumor (such as a fibrosarcoma or a carcinoma) to obtain FC. The Th cells associated with CTCL have the characteristic property of stimulating or facilitating a cytotoxic immune response after phototherapy, while the non-CTCL tumor cells (of (b) above) have characteristic tumor antigens. When FC comprising CTCL cells fused to non-CTCL tumor cells are subjected to phototherapy treatment, an antitumor response against the non-CTCL tumor is stimulated (i.e., the tumor that gave rise to the cells in (b) above) *in vivo* or *in vitro*. These fused CTCL/non-CTCL tumor cells are useful for therapeutic applications *in vitro* or *in vivo* and diagnostic assays.

As mentioned above, FC may also act as MC, i.e., migrate from a site of introduction to a second site in a patient or subject in addition to eliciting an immune response. Accordingly, FC may be "manipulated/activated" in a similar manner to MC so as to deliver a therapeutic agent/effect to a particular tissue or cell type, as discussed above in connection with MC.

Several factors are believed to be responsible for facilitating the antitumor immune response of FC. These include cytokine, histocompatibility antigen and/or adhesion molecule expression by the Th cell component of the FC. The phototherapy treatment is believed to activate the FC and/or other immune effector cells (other Th cells or B cells) in the subject. This activation underlies the antitumor response. As the specific mechanisms and molecules (lymphokine expression, Th cell activation, etc.) responsible for immune activation after phototherapy are elucidated, these can be incorporated into a cell line or population.

GMCSF, IL-4, IL-5 and/or IL-7, expressed by the Th cell component in FC, may play a significant role in stimulating an antitumor response after phototherapy. In this case, the gene(s) can be incorporated into the CTCL Th cells or the non-CTCL tumor cells prior to fusion to generate an FC population. A tumor antigen expressed by the non-CTCL cells or a histocompatibility antigen that is either under expressed or over expressed by the non-CTCL tumor cell can also be utilized in a similar manner to enhance the capacity of the FC to stimulate desired immune reactions after phototherapy treatment. The phototherapy treatment can be administered for either *ex vivo* or *in vivo* treatment of tumor cells in therapeutic or diagnostic applications.

FC can be obtained using known methods to obtain heterokaryons via fusion with appropriate effector cells. Effector cell(s) (EC) refers to either a clonal lymphoid cell or a mixture of lymphoid cells associated with an immune

with cutaneous T cell lymphoma (CTCL), (2) virus infected T cells, (3) induced

T cells involved in organ/tissue transplant rejection; and/or (4) inducer T cells involved in immune diseases. However, since the actual mechanism of immunostimulation after phototherapy is not fully understood, other tumors and normal cells may eventually be useful as the specific genes involved in these functions become known. In this aspect of the invention, cells such as malignant cells or virus infected cells are fused with EC to obtain FC. When implanted into a subject, the FC, in turn, elicit a host response against the FC that comprises an immune reaction against both the EC and the pathological cell components (e.g., antigenic cell surface antigens such as the Th antigen) present in the FC. FC can be derived from allogenic or autologous CTCL cells and fused with allogenic, autologous, or xenogenic non-CTCL tumor cells. In general, FC will be derived from allogenic or autologous CTCL cells and fused with allogenic or xenogenic non-CTCL tumor cells.

In a preferred embodiment, the FC is prepared from a T helper 2 cell line, such as Wi147. These cells can be fused with host tumor cells using conventional techniques that are well known to those of skill in the art. In another embodiment, the FC is prepared from a cell line derived from CTCL cells. These cells can be fused with host tumor cells using conventional techniques that are well known to those of skill in the art.

The pathological cell component of the FC of the invention can be an MC. However, this is not necessary. Also, as mentioned, FC can operate as MC. Again, however, this is not necessary.

Relative to the subject or cell line intended for use with the FC, EC are usually allogenic but may be autologous or xenogenic. EC are characterized by one or more of the following: (1) a selectable marker or resistance gene is optionally present (neo, gpt, DHFR, etc), (2) a susceptibility gene is optionally present (thymidine kinase (TK) usually from a herpesvirus such as HSV 1 or HSV 2, cytosinedeaminase, etc), (3) a cytokine or lymphokine is optionally present (in particular, IL-4, IL-5 or IL-7 but also including IL-1 through IL-3, IL-6, and IL-8 through IL-13, GMCSF, GCSF, MCSF, α-, β- or γ- interferon,

TNFα, TNFβ, etc, (4) treated (psoralen and light, irradiated, cytokines, antiviral nucleosides, etc) *ex vivo* prior to introduction into the subject, (5) can produce infectious viral vector or can be free of helper virus (with a virus vector that optionally contains a cytokine gene, a TK-gene, a neo gene, or the like), (6) are homogenous cell line or clones or are a mix of cells, (7) are recovered from a donor after *ex vivo* genetic engineering and reinfusion in order to select for a population that survives a few days *in vivo* then expand *in vitro* and reinfused and left in and killed off by host responses, optionally coupled with ganciclovir treatment.

Cell fusion to obtain FC can be accomplished by any method that can be used to fuse two or more different cell types, (e.g., MC and cells that mediate an immune response, EC and pathological cells, to give a multinucleate cell or heterokaryon (i.e., 2 or more nuclei are present). Suitable methods include cell fusion mediated by polyethylene glycol, liposomes, lipids, viruses (usually inactivated such as sendai virus), fusogenic proteins or peptides, etc. Where only autologous cells are used to obtain FC, the FC will comprise only subject nuclei, although the fused cells in this situation can give rise to hybrid cells comprising a gene(s) inserted into one or both of the cells used for fusion.

As will be appreciated, an FC can stimulate an immune response and/or migrate. If the FC migrates (as described above), it can be manipulated/activated in any of the manners described above for MC. Or, if the FC does not migrate, it is useful for its immune response component.

VII. *Photochemical Treatment*

As used herein, photochemical treatment refers to a treatment such as that used in phototherapy for CTCL. Thus, it is expected that phototherapy will be useful for treatment of FC. An exemplary photochemical treatment

(about 20-150 ng/mL with 50, 50, 5 and 100 50 ng/mL as typical doses),

followed (about 20-120 minutes later) by dosing with UVA at about 0.5 - 2.5 joule/cm2 (can be 0.75, 1.0, 1.5 and 2.0 joule/cm2). 8-MOP can be delivered to lymphocytes by administration *in vivo* to the donor or *ex vivo* to lymphocytes obtained from the donor. Optimal doses of 8-MOP and UVA can be determined empirically through routine experimentation. For example, a dose response curve can be prepared to measure inhibition of lymphocyte proliferation (Edelson et al. *N Engl J Med* **316**:297 (1987)) or other well known protocols (Edelson *Ann New York Acad Sci* **636**:154 (1991), Knobler et al. *Ann New York Acad Sci* **636**:340 (1991), Berger et al. *Ann New York Acad Sci* **636**:266 (1991), Perez et al. *Ann New York Acad Sci* **636**:95 (1991), and Bisaccia et al. *Ann New York Acad Sci* **636**:321).

It should be noted that in some instances, it may be preferable or necessary to photolyze peripheral blood lymphocytes at the same time as FC. Such lymphocytes may secrete cytokines or other agents that could boost the therapeutic index or the immune response level.

VIII. *Lethal Or Sublethal Irradiation/DNA Synthesis Inhibitors*

Lethal radiation of cells, as used herein, refers to irradiation, usually a dose of gamma irradiation, sufficient to kill irradiated cells by preventing DNA replication. Such cells usually remain "alive" for a period of several days (or even a week to 10 or more days) and can migrate and retain other functions such as the capacity to synthesize proteins and transport proteins to the cell surface after gamma irradiation treatment. However, such cells will not be able to replicate and, when implanted into a subject *in vivo*, cannot usually survive for extended periods of time.

For example, through generally using from about 1500 to about 5000 rads of gamma or X-irradiation radiation, cells can be successfully prevented from undergoing DNA replication. More preferably, from about 2500 to about 4000 rads of radiation is used. In highly preferred embodiments 2500, 3000,

and 3500 rad doses of radiation are used to lethally irradiate the cells prior to implanting the cells into a subject to be treated.

Somewhat lower doses of radiation can be used for sublethal irradiation. Typically in sublethal irradiation gamma or x-radiation on the order of 500 to 1500 rads is used. For example, a dose of approximately 1000 rads will kill T cell response and leave humoral response intact.

As an alternative to lethal irradiation, cells may also be inactivated, prior to implantation into a subject, by chemical treatments. For example, DNA synthesis inhibitors can be used such as mitomycin C and the like that will allow the cells to operate but not replicate.

IX. *Gene Insertion Techniques*

Any known method to introduce genes is acceptable to deliver a gene or genes into target cells, such as lymphocytes, malignant cells, myoblasts, hepatocytes, endothelial cells, epithelial cells, chondrocytes, glial cells, astrocytes, stem cells of intestinal epithelium (WO 93/19660), cord blood cells (stem cells, white blood cells and lymphocytes), fetal and postnatal central nervous system cells, neurons, virus infected cells and the like, where such gene(s) are expressed at least transiently (at least 12-24 hours). Exemplary means of gene transfer to target cells includes the use of retroviral vectors, transfection (via calcium phosphate, dextran sulfate and the like) or electorporation of naked DNA such as virus/expression vectors, adenoviral vectors, herpesvirus vectors (WO 93/19591), cell fusion liposome or cationic lipid mediated transfection and the like. Direct transfer of genes and viral vectors *in vivo* and *ex vivo* has been described and can also be utilized to transfer genes to MSC *in vivo* (Nabel *Proc Natl Acad Sci USA* **90**:10759 (1993); Bandara *Proc Natl Acad Sci USA* **90**:10764 (1993)).

into the MSC. Usually gene transfer will be accomplished in tissue culture, but

is also specifically intended to include protocols where (1) gene transfer to MSC is accomplished *in vivo* or (2) gene transfer to other cells *in vivo* (e.g., tumor cells found near MSC after MSC have migrated to the resident tumor cells in a subject) by MSC genetically altered cells to produce retroviral vector particles. *In vivo* gene transfer using retroviral vectors and other means is discussed in, for example in WO 93/02556, the disclosure of which is incorporated by reference. Gene transfer for human therapeutic applications requires the use of vector stocks that do not contain replication competent viruses. However gene transfer in the present invention is also intended to include gene transfer protocols that use producer cell lines or virus stocks (i.e., supernatants or concentrates containing the virus vector) containing replication competent virus for gene transfer *in vivo* or for gene transfer *in vitro* in either human or nonhuman subjects.

Virus stocks containing replication incompetent virus can have relatively low titers of incompetent virus, i.e., vector, (1×10^1 to 1×10^4 competent particles or plaque forming units per mL or any titer of virus particles in this range in increments of 2×10^2 competent particles or plaque forming units per mL) or the titers can be relatively high (about 1×10^5 to 1×10^8 vector particles or plaque forming units per mL or any titer of virus particles in this range in increments of 1×10^5 competent particles or plaque forming units per mL).

Virus stocks containing replication competent virus can have relatively low titers of competent virus (1×10^2 to 1×10^4 competent particles or plaque forming units per mL or any titer of virus particles in this range in increments of 2×10^2 competent particles or plaque forming units per mL) or the titers can be relatively high (about 1×10^5 to 1×10^8 competent particles or plaque forming units per mL or any titer of virus particles in this range in increments of 1×10^5 competent particles or plaque forming units per mL).

The use of stocks containing replication competent virus will facilitate the spread of virus vector particles. Vector particles are usually replication defective. In general, when used in human subjects, virus stocks containing

replication competent vectors will not be used where the immune system of the subject is weak or suppressed. For example, the immune system in patients with an HIV infection is generally considered weak and suppressed in patients after a transplant. Avoiding the use of replication competent vectors in such patients will limit the chance of causing an infection that cannot be controlled by the immune system and/or by antiviral therapy.

The spread of a vector from MC to nearby cells *in vivo* is useful for enhancing the spread of a therapeutic gene (and hence its *in vivo* effect) such as TK or a lymphokine such as IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, GCSF or GMCSF and the like. The spread of replication incompetent or replication competent virus and associated virus vectors (i.e., a vector derived from the same or a closely related virus), such as those derived from adeno-associated virus (Chatterjee *Science* **258**:1485 (1992)), adenoviruses, human herpes viruses (WO 93/19591), murine leukemia viruses (Freeman *Cancer Research* **53**:5274 (1993); Culver et al. *Science* **256**:1550 (1992), murine spleen necrosis virus, gibbon ape leukemia virus and the like, *in vivo* will generally be limited by the subject's natural defenses (particularly where the subject is a species different from the normal host for the virus) including immune responses to the virus or by natural defenses in combination with antiviral chemotherapy.

Thus, in accordance with the invention, adenoviral and retroviral facilitation of incorporation and therapy are contemplated. Also, adenoassociated viral vectors have shown great promise in therapeutic applications are also contemplated. Further, other delivery and incorporation aiding techniques are also suitable. Principally, these techniques include the use of liposomes and DNA conjugates are expected to provide similar delivery yields as those provided by the viral vectors discussed above. In addition combination therapies of adenoviruses and liposomes have also shown

which is hereby incorporated by reference.

Liposomes are known to provide highly effective delivery of active agents to diseased tissues. For example, pharmacological or other biologically active agents have been effectively incorporated into liposomes and delivered to cells. Thus, constructs in accordance with the present invention can also be suitably formed in liposomes and delivered to selected tissues. Liposomes prepared from cationic lipids, such as those available under the trademark LIPOFECTIN (Life Technologies, Inc., Bethesda, MD) are preferred.

Particularly appealing to liposome based treatments is the fact that liposomes are relatively stable and possess relatively long lines, prior to their passage from the system or their metabolism. Moreover, liposome do not raise major immune responses.

Thus, in one aspect of the present invention a vector containing a construct of the invention is incorporated into a liposome and used for the delivery of the construct to a specific tissue. The liposome will aid the construct in transfecting a cell and causing the cell to express protein.

In another aspect, the liposomes containing the constructs may be directly injected into a patient, such as into the patient's liver, and therapy will thereafter commence.

DNA conjugates are newcomers to the genetic therapy delivery art. DNA conjugates typically contain a mass of DNA containing one or more expressible vectors coupled with a polyionic compound. In addition, the conjugates may be coupled to a transferon molecule, a transferon-like molecule, or other promotion factor. Still further, the conjugates can also be coupled with a viral capsid.

Similar modes of treatment are contemplated for DNA conjugates as those discussed above.

X. *Dosing*

Where therapeutic uses for an MC or MSC in a given human or nonhuman subject (mouse, rat, human, monkey, rabbit, horse, dog,cat, etc) are

contemplated, details of the protocol to be used will be obtained by routine methods for administering and using cell lines in subjects. For example, doses of MSC to be administered to a human will be determined by dose escalation studies using relatively low cell numbers (about 1 x 10^7 to about 1 x 10^9 MSC) in the subject, followed by escalating doses (about 2 x 10^9, 5 x 10^9, 1 x 10^{10}, 2 x 10^{10}, 4 x 10^{10}, 1 x 10^{11}, 2 x 10^{11}, 1 x 10^{12} cells) in the same subject or another subject of the same or similar species.

Generally, about 1 x 10^8 to 2 x 10^{11} MSC or any number of MSC within this range in increments of 1 x 10^8 or 2 x 10^8 will be used in a human subject to elicit a therapeutic response. Preclinical work in non human subjects (i.e., mice, rats, rabbits, guinea pigs, dogs, monkeys, etc.) will usually precede any clinical use of MSC in humans. MSC administered to nonhuman subjects will be adjusted accordingly for the size of the animal and the route of cell administration. Small animals such as mice, guinea pigs, or rats bearing tumors or having viral infections can be treated using about 1 x 10^4 to 1 x 10^7 MSC or any number of MSC within this range in increments of 2 x 10^4 or 2 x 10^5 MSC cells, with about 1 x 10^5, 5 x 10^5 or 1 x 10^6 MSC/treatment being exemplary.

For MSC that contain the TK gene, a nucleoside analogue (i.e., ganciclovir or acyclovir) will usually be administered to the subject at the same time or shortly after (1-48 hours, although longer time periods can be used such as 3, 4 or 5 days up to about 8 days) the MSC are administered to the subject. The nucleoside analogue is administered to subjects by conventional means including intravenous injection or oral doses. In the case of ganciclovir or acyclovir, typical doses range from 5-250 mg/Kg, or any dose between these values in 1 mg/Kg increments, administered 1, 2 or 3 times per day for a period of from about 2 to about 8 days in order to effect killing of TK+ MSC.

XI. *Alternative Therapeutic Embodiments*

In one embodiment, MSC are genetically engineered to contain and express both the TK and granulocyte/macrophage colony stimulating factor (GMCSF) genes. Such MSC can be obtained from, for example, human or mouse donor cells (including cells from a human or mouse cell line) and used to treat a tumor in a subject such as a mouse, rat, horse or human. Such cells can be selected for *in vivo* migration using a subject such as a mouse, rat or human. In addition, the genetically-engineered MSC can be lethally irradiated by routine methods, as described above.

Another embodiment of the invention is the use of MC containing the interleukin 6 (IL-6) gene along with the TK gene. In one aspect of this embodiment, the IL-6 is useful for stimulating platelet production in human or nonhuman subjects undergoing cancer chemotherapy or treatments, for example in human small cell lung carcinoma, in sarcoma treatments in human or nonhuman subjects or in other cancer protocols in human or nonhuman subjects. In other aspects, MC containing IL-6 and TK genes are used to facilitate reconstitution of transplanted marrow in leukemia treatments in human or nonhuman subjects.

In another embodiment, MC containing the GCSF and TK genes are used in marrow transplantation in breast cancer treatment. The GCSF expressed by the MC *in vivo* facilitates reconstitution of the marrow after transplantation. The TK gene serves to allow killing of the MC *in vivo* by administering ganciclovir to the subject carrying implanted MC.

In another embodiment, MC containing IL-6 or GCSF and the TK gene are administered to a subject along with administration of chemotherapy, cytokines or other treatments (such as monoclonal antibodies that bind to tumor antigens such as CD20 associated with B cells and B cell lymphomas in humans) to the subject. About 10 to 500 milligrams of antibody per square meter of body surface are administered to a human subject by infusion over a period of several hours (2 to 4 hours generally) or over a shorter period for

nonhuman species. Optimal dosing and administration protocols will be derived by standard means including dose escalation studies for MC, ganciclovir (for TK+ MC), chemotherapy, lymphokines and/or antibodies as appropriate.

During treatment of a cancerous condition in a human or nonhuman subject (cancer is meant to include conditions in human, rodent, nonrodent, primate or nonhuman subjects including such as ovarian carcinomas, breast carcinomas, leptomeningeal carcinomatosis, colon carcinomas, glioblastoma, a B or T cell lymphoma, a B or T cell leukemia, neuroblastoma, soft tissue sarcomas, cervical carcinoma, lung carcinomas, urinary bladder carcinomas, stomach carcinomas, adrenal cortex carcinomas, endometrial carcinoma, prostate carcinoma, fibrosarcomas, adenocarcinomas, pancreatic carcinomas, biliary duct carcinomas, liver carcinomas, and the like, the dosing and administration of MSC, radiation therapy, chemotherapy (such as treatments with DNA alkylating agents, steroids and/or nucleoside analogues including anthracyclines, chlorambucil, melphalan, hexamethylmelamine, methotrexate, semustine, dacarbazine, 5-fluorouracil, cytarabine, 6-mercaptopurine, vinblastine, doxorubicin, bleomycin, mitomycin C, cis-platin, hydroxyurea, tamoxifen, testosterone propionate and the like), lymphokines, antibodies and the like will be adjusted according to the condition of the subject and its response to the therapeutic treatment regimen.

For example, if a patient with say, ovarian cancer relapses after conventional chemotherapy or fails to respond adequately to chemotherapy, greater reliance on TK+ MC derived from the subject's own ovarian cancer cells (or from another allogenic or xenogenic source of cancer cells) will be indicated. Examples of other allogenic or xenogenic cell lives that are useful as MC include murine fibrosarcoma cells (for example, American Type Culture Collection Catalog No. CCL 163), murine mammary tumor cells (for example, EMT 6.8 cell line [illegible]

ovarian adenocarcinoma cells (for example, American Type Culture Collection

SK-OV-3 cell line), murine myelomonocyte tumor cells (for example, American Type Culture Collection No. TIB 68; the WEHI-3 cell line) or human colon carcinoma cells (for example, American Type Culture Collection No. GCL 225).

As described above, an aspect of the invention is the use of MC for treatment of malignant cells derived from a first subject, such as a human that are implanted into a second subject such as a mouse or rat. In this embodiment, the MC can be used in combination with other treatments (chemotherapy, radiation therapy, etc.) in order to screen for the efficacy of new potential therapeutic agents or to screen for the efficacy of new combinations of conventional agents in combination with lymphokines or cytokines using MSC such as TK+ MSC with or without lymphokines or cytokines.

Another embodiment utilizes hematopoietic (such as autologous or allogenic human or nonhuman CD34+ cells) or other stem cells as donor cells for generating MC. Such MC can be genetically engineered with, for example, the TK gene (as a means to select cells after explant from a subject) and optionally other genes such as lymphokines or cytokines. When implanted into a patient, such MC are permitted to migrate *in vivo* followed by administering a treatment to the subject that facilitates formation of differentiation of the MC.

Such MC can facilitate reconstitution of implanted MC. In this embodiment, MSC that are not differentiated are obtained from a subject after 2, 3, 4 or more rounds of migration/selection. It is to be understood that in the present invention, treating a disease by administering MC/MSC, and optionally other therapeutics to a human or nonhuman subject, is intended to prevent, ameliorate or eliminate a condition (e.g. to eliminate or ameliorate a virus infection such as HIV in human or nonhuman subjects such as the SCID mouse or in primates, murine leukemia virus infection in the mouse or other subjects, human or murine CMV, HSV-1 and the like, or cause cancer such as melanoma, glioma, neuroblastoma, breast carcinomas, leptomeningeal

carcinomatosis, ovarian cancer or the like to go into remission temporarily or permanently).

The objects of MC treatments also include causing a reduction in episodes associated with organ or cell rejection in transplants, restoring normal control to hyperproliferating Th cells and treating either an established disease (retrovirus infection, melanoma, ovarian cancer, CTCL, SLE) or treating prophylactically, as for organ or tissue transplant pretreatments.

In a similar manner, the invention can also be applied to the treatment or amelioration of autoimmune diseases. Treatment of autoimmune diseases or diseases with an autoimmune component has been described. Heald *Ann New York Acad Sci* **636**:171 (1991), which together with its cited references is hereby incorporated herein by reference. MC that will target a subpopulation of cells that are responsible for autoimmune attack can be used to treat an established autoimmune disease or can be used to prophylactically protect against the onset of an autoimmune condition.

It is thus intended that the treatments are not always required to effect a complete cure of a condition in a subject in order to be successfully applied.

Another embodiment of the invention includes the use of retroviral vectors to transfer a drug susceptibility gene into MC. Exemplary vectors include STK, μTK, pIL-1, LASN and LNL as previously described (WO 93/02556). The STK vector carries genes that encode the neomycin phosphotransferase gene (Neo) from the bacterium *Escherichia coli* and the human herpes simplex virus thymidine kinase gene (TK). TK genes from other viral sources could be utilized in place of the TK gene in STK in those cases where the enzyme confers nucleoside analog susceptibility on TK+ cells relative to TK- cells. The neo gene was expressed by the vector's LTR promoter and the TK gene was expressed by an SV40 virus early promoter as

a suitable nucleoside analog such as ganciclovir. In this embodiment, the STK

and μTK vectors are used to transfer a herpesvirus TK gene into MC in order to generate TK+ MC including MC derived from malignant cells, hematopoietic stem cells or from T lymphocytes.

Cells carrying the neo gene are also resistant to killing by the neomycin analog G418, which is toxic to eukaryotic cells that do not contain an immunoglycoside resistance gene, such as mammalian cells that do not express the neo protein. The neo gene permits growth of cell populations wherein the cells contain and express the vector by growing the cells in the presence of G418. This ensures that all cells that lack the vector are killed before subsequent studies are carried out in subjects. The LNL vector carries only the neo gene and serves as a control to show that the effects observed in subjects are not due to the presence of either vector sequences or to the presence of the neo protein.

The μTK vector is similar to the STK vector except that the TK gene is expressed from the herpes virus TK promoter. Any retroviral vector carrying a herpes virus TK gene is suitable for generating TK+ MC. Expression vectors, including retroviral and adenoviral vectors, used to generate TK+ MC, will optionally contain an additional gene such as a cytokine (human, simian, murine, porcine or bovine growth hormone, human or murine basic or acidic fibroblast growth factor, transforming growth factor α or β angiogenesis factors and the like), a lymphokine (human or primate IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, GMCSF, GCSF, MCSF and the like; murine IL-2, IL-3, IL-4, IL-6, IL-10, GMCSF, GCSF, MCSF and the like), other human or nonhuman hormones including human, murine, simian or porcine hormones or another gene as indicated for a given application, assay or treatment.

Also included in the present invention are embodiments where more than one expression vector such as DNA carrying a gene(s) to be expressed, adenovirus vectors or retrovirus vectors (or combinations thereof, including naked DNA and a retrovirus vector, anadenovirus vector and a retrovirus vector) are used to generate a given genetically-engineered MC line or

population. For example, a first expression vector carrying a TK gene can be introduced into MC along with a second vector carrying a selectable marker gene and/or a lymphokine or cytokine can be used. Situations where two expression vectors are indicated include those requiring the expression of a large DNA (about 9, 10, 11, 12, 13, 15, 20, 30, 40, 50, 2560 or more Kilobases in length), such as a large cDNA or a gene containing introns, and another gene (lymphokine, cytokine, growth factor, blood coagulation factor etc). In these cases, existing viral vectors may not be capable of carrying gene inserts of sufficient length. Therefore, use of a plurality of vectors may be necessary to prepare transduced (i.e., activated) MC and are intended to be included in discussions of transductions or transfection of cells.

In some embodiments, an aspect of the present invention is the homing of MC to similar or identical donor cells *in vivo*. A cell-mediated response is believed to be responsible for immunization of animals after challenge with TK+ MC followed by their elimination by, for example, ganciclovir killing of TK+ MC and donor cells *in vivo* within a few days after ganciclovir is administered shows that another mechanism is responsible for tumor cell killing. The rapid cell killing after ganciclovir therapy is initiated shows that subject pathological cell (such as tumor cells) killing responses occurs before known cell mediated responses are established. The donor cell killing is due to homing of TK+ MC to resident donor (such as tumor) cells.

The precise nature of the MC killing response by the subject is not fully understood at a molecular level, but, in some cases, involves homing of MC to donor cells in the subject. The ability of some cell types including malignant cells to migrate or metastasize is known as is the phenomenon whereby cells of the same type tend to aggregate or grow together both *in vitro* and *in vivo*. In the present case, TK+ MC migrate to donor cells (such as tumor cells) resident

gene such as a cytokine or a lymphokine (i.e., GMCSF or an interleukin) which will potentiate the therapeutic index.

In some embodiments, MC derived from cytotoxic T cells, dendritic cells that have cytotoxic activity against tumor antigens such as the carcinoembryonic antigen, or the human mucin (muc-1) gene are obtained. Such MC are obtained from a human or nonhuman subject having a cancer such as breast, prostate, pancreas, colon or certain renal cell carcinomas and are optionally genetically engineered to contain TK and/or a lymphokine such as GMCSF, IL-2, IL-4 and/or IL-6. MC can be selected for enhanced migration through *in vivo* or *in vitro* techniques, as described above, to obtain MSC. MC can be transduced before or after selection. The MC/MSC are then delivered to the subject having the cancer in order to effect an antitumor response.

Such MC tumor vaccines can also be derived from nontumor cells (allogenic or xenogenic) which are more immunogenic than tumor cells. MC derived from such nontumor cells are genetically engineered to contain a tumor antigen, a lymphokine (IL-2, IL-4, GMCSF) and/or TK.

In view of the foregoing discussion, it is clear that a substantial number of genes or types of genes can be inserted into donor cells or into MC in order to obtain useful genetically-engineered MC for therapeutic, diagnostic or assay purposes. Exemplary groups of genes that can be used to generate genetically-engineered MC include (or can exclude any subset or individual of) the following:

1) cytokines or growth factors including (or can exclude) transforming growth factor β-3, transforming growth factor β, insulin-like growth factor 1, growth hormone (human, bovine, porcine, murine, simian and the like);

2) lymphokines (human IL-1 through IL-13, GMCSF, GCSF, MCSF; murine IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, GMCSF, GCSF, MCSF, Rantes and other inflammatory stimulators; analogous lymphokines from other subject species);

3) angiogenesis factors;

4) serum proteases such as thrombin, factor VIII, factor IX, factor X, a protease in the complement fixation pathway;

5) intracellular metabolic enzymes which include (or can exclude) adenosine deaminase, proteases, cytosine deaminase, glucocerbrosidase;

6) cell surface proteins or antigens including (or can exclude) lymphocyte surface antigens including CD1, CD1a, CD4, CD5, CD7, CD8, CD20, CD34, T cell receptor, cell adhesion molecules including ELAM-1, ICAM-1, VCAM-1, E-slectin, receptor molecules including (or can exclude) IL-1 through IL-13 receptors, GMCSF receptor, GCSF receptor, MCSF receptor, neurotransmitter receptors, ion channels or subunits thereof including (or can exclude) chloride channels, potassium channels, extracellular matrix molecules;

7) tissue-specific gene products including (or can exclude) globin, myosin, neurotransmitors, bilirubin conjugators;

8) tumor antigens including (or can exclude) the carcinoembryonic antigen, melanoma associated antigens and the like;

9) histocompatibility antigens;

10) virus antigens including (or can exclude) the envelope or nucleocapsid of HIV, SIV, human or murine cytomegalovirus, HSV-1, HSV-2, human papilloma viruses (serotypes 1-55);

11) cell surface antigens of parasitic protozoa including malaria sporozoites, trypanosome sporozoites and the like; and

12) genes from plants such as cellulase.

XII. *Diagnostic Applications*

Another use of the present invention is for diagnostic purposes. The

administration (e.g., injection) with markers that can be detected in the subject

such as radiolabels (i.e., radionuclides) or genetic marking. Microscopic tumor deposits will then be detectable through detecting the MC cells that have migrated/homed to the microscopic deposits which are very difficult to detect by other methods.

An advantage to the use of MC in this diagnostic procedure is that MC are not as susceptible to the rapid clearance patterns seen for antibodies (i.e., monoclonal antibodies and polyclonal antibodies). Further, the degree of nonspecific binding observed for MC as compared to antibodies is significantly lower

Thus, MC labeled with a known suitable emitter such as ^{121}I or ^{131}I or a suitable radio contrast agent are administered to a host, preferably through injection, followed 2-4 days later by imaging the subject to locate the injected MC. Methods for full body scanning of patients or subjects for detecting radioisotope or other detectable mediums are well known in the art. Fluorescently labeled MC derived from allogenic tumor cells have been found to migrate to tumors resident in a subject.

MC derived from a particular donor cell line or population will usually retain many of the characteristic properties of the donors. For example, tumor cells used to generate MC will retain their capacity to generate tumors when implanted into a susceptible subject *in vivo*. MC derived from hematopoietic precursor or stem cells of various types will retain their capacity to differentiate normally *in vivo* or *in vitro* in response to appropriate signals or growth conditions. MSC derived from endothelial cells will retain their capacity to participate in angiogenesis or in other vascular functions, such as facilitating clot formation or inflammatory reactions.

However, in some cases, MC will be selected that have one or more characteristic properties that differ or are enhanced or diminished from the donors such that the cells can more effectively migrate when injected into the host. Such MC can be intentionally selected using appropriate subjects and/or migration/selection conditions. For example, MC prepared from tumor cells

may be more immunogenic than the original population of tumor cells. MC derived from CD34+ cells may have a greater likelihood of being reseeded in the bone marrow after infusion. MC selection may be performed in a host other than the one that is used for selection (i.e., allogenic or xenogenic). For example, human tumor cells may be injected I.V. into a mouse that has a subcutaneous tumor consisting of the human tumor cells or other human or non-human tumor cells. After selecting for MSC, those cells may be used therapeutically by injecting them into humans.

XIII. *Illustrative Descriptions*

Embodiments of the invention include the following illustrative descriptions:

A method to obtain a cell population comprising the steps of: (a) explanting cells from a first subject to obtain a donor cell population; (b) preparing a single cell suspension of the donor cell population (by, say, treatment with protease such as trypsin or by mechanical disruption of the cells); (c) optionally infecting the single cell suspension with a viral vector (such as adenoviral or retroviral vector) or transfecting the single cell suspension with an expression vector; (d) preparing the single cell suspension for implantation into a second subject; (e) implanting the cells of step (d) into the second subject that has optionally been sublethally irradiated to immunosuppress cytotoxic responses of the second subject; (f) allowing the implanted cells to migrate to a second site (such as a tumor, lymph node, lymph duct, spleen, thymus, liver, artery, vein, a site associated with virus infected cells or a site associated with an autoimmune response such as the pancreas); (g) recovering the implanted cells from the second site; and optionally repeating steps (b)-(f) 1, 2, 3, 4, or 5 times.

[illegible] growth of the donor cells of the MC MSC *in vitro*. Variations of this

embodiment can include maintaining explanted MSC in tissue culture for certain manipulations (i.e., insertion of a gene(s), etc.) after the first round of migration/selection *in vivo*. Maintaining explanted MSC in tissue culture for a short period can be useful for MSC (such as certain cell types including human or nonhuman hepatocytes or hepatocyte stem cells) that are difficult to grow in tissue culture.

A method to obtain a cell population comprising the steps of: (a) treating human CD34+ hematopoietic cells with TNF-α and IL-3 or with GMCSF *in vitro*; (b) optionally genetically engineering the CD34+ cells; (c) implanting the CD34+ cells into an autologous or xenogenic subject; (d) isolating CD34+ from the subject to obtain CD34+ MSC; (e) growing the CD34+ MSC *in vitro* in the presence of TNF-α and IL-3 or with GMCSF; (f) optionally repeating steps (c)-(e) 1, 2, 3, 4, 5 or 6 times; (g) isolating CD34+ MSC that express the CDla+ antigen to obtain CDla+ MSC; (h) optionally growing the CDla+ MSC *in vitro* in the presence of TNF-α and IL-3 or with GMCSF; (i) optionally generating CD4+ T cells from the CDla+ MSC; (j) optionally using the CDla+ MSC to generate cancer-specific or virus-specific CD8+ cytotoxic T cells; and (k) optionally implanting the CD1a+ MSC into an autologous or xenogenic subject followed by recovering the CD1a+ MSC and expanding the recovered cells *in vitro*.

In step (i), the generation of CD4+ T cells from the CDla+ MSC is accomplished by using the CD1a+ MSC to generate cancer-specific or virus-specific CD8+ cytotoxic T cells. In this embodiment, CD34+ cells are obtained and grown as described (WO 93/20186). CD34+ MSC are obtained, for example, by *in vivo* migration/selection in a mouse or rat (such as a nude mouse, a sublethally irridiated mouse or rat where cytotoxic immune responses are reduced or eliminated, or in a mouse or rat immunosuppressed by treatment with, say, cyclosporin A or with FK-506). Multiple rounds of migration/selection can be conducted in the same manner with CDla+ MSC.

In a variation of this embodiment, CD34+ cells are obtained by *in vivo* migration/selection in an autologous subject and the CD34+ MSC are obtained from a location such as a tumor, lymph node, lymph duct, peripheral blood, autologous cord blood, a site associated with an autoimmune response, white blood cells associated with virus infected cells or from virus infected cells (lymphocytes and the like). Subsequent rounds of migration/selection using CDla+ MSC are carried out in an autologous subject or a xenogenic subject such as a SCID-hu mouse. Where possible, autologous cord blood is used as a source of CD34+ donor cells.

CD34+ MSC or CDla+ MSC can be used in a mixed lymphocyte reaction (MLR) comprising the steps of: (a) providing responder cells suitable for MLR; (b) providing inactivated allogenic CD34+ or CD1a+ MSC; (c) coculturing the responder cells and the inactivated cells; and (d) measuring a response of the responder cells such as responder cell proliferation via measuring incorporation of ^{3}H-thymidine by responder cells.

In another embodiment, MSC implanted in a subject migrate to a second site, followed by transfer of a gene(s) into the MSC *in vivo*. In this is embodiment, donor cells (or MSC derived from such cells) that are difficult to genetically engineer *in vitro* or that are difficult to grow in tissue culture, are genetically engineered *in vivo* by any suitable means including transfection with liposomes containing DNA **(citation)**, infection with virus vectors **(citation)** or transfection with DNA-cationic lipid complexes **(citation)**. This embodiment provides a method comprising the steps of: (a) introducing donor cells or MC into an autologous, allogenic or xenogenic subject; (b) allowing the donor cells or the MC to migrate to a second site; (c) genetically-engineering, infecting or transfecting the donor cells or MC *in vivo* by a suitable means; (d) and optionally explanting the donor cells or MC to detect the presence, absence or

[illegible] explanted cells. In this and in other embodiments, is accomplished by any of

a number of well known methods including (a) PCR amplification of unique DNA or RNA sequences that were inserted into donor cells or into MC prior to implantation into a subject, (b) fluorescence activated cell sorting or flow cytometry for MC expressing suitable gene products such as surface antigens that can be fluorescently labeled, using for example antibodies or monoclonal antibodies (labeled with fluorescein isothiocyanate, texas red, rhodamine or the like) or using cytoplasmic enzymes (such as β-galactosidase and the like) that can generate a fluorophore from a suitable substrate (such as X-gal), or (c) detecting the presence or amount of MC gene products (such as a lymphokine and the like) or nucleic acid sequences by biological assay, enzyme-linked immunosorbent assay, radioimmunoassay or by western or southernblotting.

Methods to conduct such assays are well known (see for example: Current Protcols in Molecular Biology F. M. Ausubel et al. eds. (Wiley (1989)), vols 1 & 2, and supplements, see especially chapters 2, 4, 9, 10, 11, 12, 14, 15, 16; Molecular Cloning: A Laboratory Manual J. Sambrook et al. eds. (2d Edition (1989)).

When MC in an allogenic or xenogenic subject are to be detected or isolated, protein or nucleic acid sequence differences between genes or alleles of genes between the MSC and the subject are useful to facilitate detection or isolation of the explanted MSC.

In embodiments where donor cells from a first subject species such as a human or monkey are used to generate MSC in a second subject species such as a mouse, rat or dog, multiple rounds of migration/selection will optionally be carried out in closely related, or genetically identical individuals of the second species. In the case of mice, syngenic or genetically identical individuals can be used for each round of *in vivo* migration/selection. In a second species such as the dog, litter mates can be used, while in other species, such as the monkey, allogenic individuals can be used for each round of *in vivo* migration/selection. It is also intended that multiple rounds of *in vivo* migration/selection can be carried out in a single individual of a second species

such as a dog, rabbit, monkey or the like. For example, in a relatively large second species such as dog or monkey, MSC can be obtained by surgical excision or biopsy of cells from a second site such as an organ or tumor on several successive occasions.

The methods used to obtain MSC from a second species will be guided by common sense parameters that are apparent to one skilled in view of the present disclosure. Such parameters include (a) the number of MSC that must be explanted in order to grow MSC, usually *in vitro*, (b) suitable methods for distinguishing MSC from donor cells such as genetically marking donor cells with a selectable gene prior to implantation into the second subject, (c) methods that are available to grow donor or MSC *in vitro* or *in vivo*, and (d) the number of donor cells that must be implanted in a second subject in order to obtain sufficient MSC at the second site for further manipulations.

Each of these considerations can be addressed by application of known techniques for culturing, implanting, recovering and detecting specific cells in a population.

The present invention is described in more detail by the following nonlimiting illustrative examples:

EXAMPLE 1

IN VIVO RESPONSE TO GANCICLOVIR THERAPY USING Kbalb-STK AND Kbalb-LNL CELLS

In this experiment, we first conducted an *in vitro* ganciclovir sensitivity test followed by an *in vivo* tumor regression assay. The Kbalb murine fibrosarcoma cell line was transduced with the STK vector and grown in standard tissue culture in order to select for a population of cells that all carry and express the STK vector. Transduction was accomplished as described in WO 93/02556 (Example 1).

clone AST cell line (ATCC No CCL 163). STK vector stocks were obtained

from the Ψ2 retroviral packaging line. 150 cells were plated on 10 cm tissue culture dishes 18 hours prior to exposure to DMEM containing either 1 μM or 10 μM ganciclovir for different periods of time. Medium was then removed and the plates were washed three times with sterile phosphate buffered saline to remove ganciclovir. Thereafter, fresh DMEM without ganciclovir was added.

Colonies were then counted 14 days later. The results demonstrate that the Kbalb-STK cell line was sensitive to killing by a brief exposure to ganciclovir.

Four groups of four BALB/c mice per group were injected subcutaneously (s.q.) with 2 x 10^5 cells in sterile saline per animal using the following cells or cell mixtures:

Group 1: Kbalb-STK;

Group 2: 90% Kbalb-STK and 10% Kbalb-LNL;

Group 3: 50% each Kbalb-STK and Kbalb-LNL; and

Group 4: 100% Kbalb-LNL.

Beginning three days after injection, ganciclovir was administered i.p. twice daily at a dose of 150 mg ganciclovir/kg body weight for 5 days. Tumor size at the injection site was determined from one animal from each group on days 0, 3, 9 and 17 from the time the cells were injected into the animals.

The results shown in Table 1 indicate tumor growth occurred in all groups until day three. After initiation of ganciclovir therapy, tumor size on day 9 remained the same as at day three and had regressed completely by day 17 in Groups 1, 2 and 3. Group 4 tumor growth continued unchecked throughout the duration of the experiment.

TABLE 1

Animal Group	Day After Injection	Tumor Diameter (mm)
1	0	0
	3	2.5
	9	2.2
	17	0
2	0	0
	3	2.3
	9	2.3
	17	0
3	0	0
	3	2.4
	9	2.4
	17	0
4	0	0.0
	3	2.4
	9	8.0
	17	14.2

These results clearly showed killing of both ganciclovir sensitive and ganciclovir resistant tumor cells *in vivo* in animals having tumors that consisted of resistant and sensitive cell mixtures.

EXAMPLE 2

IN VIVO RESPONSE TO GANCICLOVIR THEREAPY USING Kbalb AND EMT 6.8 CELL POPULATIONS TO GENERATE A PRE-EXISTING INTRAPERITONEAL TUMOR AND TREATMENT WITH TK-MODIFIED Kbalb

In this experiment, we were interested in determining whether a mammal having a pre-existing tumor could be treated through homing a TK-modified tumor cell to the exisiting tumor followed by ganciclovir therapy. Accordingly, we targeted Kbalb-STK cells to resident Kbalb-LNL or EMT 6.8 tumors intraperitoneally.

The Kbalb-LNL and Kbalb-STK tumor cell lines were obtained as in Example 1. The EMT 6.8 cell line is a murine mammary tumor cell line and was obtained from Dr. Edith Lourde at the University of Rochester.

Six groups of six BALB/c mice per group were injected i.p. on day 0 with 2 x 10^5 transduced tumor cells in sterile saline per animal as follows:

Group 1: Kbalb-LNL;

Group 2: Kbalb-STK;

Group 3: 50% each Kbalb-STK and Kbalb-LNL;

Group 4: Kbalb-LNL (followed 24 hours later with 1 x 10^6 Kbalb-STK cells were injected i.p.);

Group 5: EMT 6.8; and

Group 6: EMT 6.8 (followed 24 hrs. later with 1 x 10^6 Kbalb-STK).

The initial injection, indicated, was used to form the tumor in the animals. The second injection, where indicated, was used as the targeting cell or MC. On day five, 5 days after initial cell injections for all groups, daily ganciclovir therapy (150 mg ganciclovir/kg mouse, injected i.p., twice daily) was administered.

Survival of the animals was then followed over time. The LNL vector carries only the neo gene and serves as a control to show that the effects observed in the subjects are not due to the presence of either vector sequences or to the presence of the neo protein. The results for Groups 1-4 are shown in Table 2.

TABLE 2

Animal Group	Day After Injection	No. Surviving Animals
1	15	6
	16	4
	17	2
	18	0
2	15	6
	16	6
	17	6
	18	6
3	15	6
	16	6
	17	6
	18	6
4	15	6
	16	5
	17	5
	18	5

The results shown in Table 2 (number of animals surviving in each

compared to controls (Group 1). 5 out of 6 Group 4 animals survived over the

30 day time period of the experiment. The result from Group 4 demonstrated that transduced tumor cells introduced into an area near a pre-existing tumor leads to regression of the pre-existing tumor after appropriate therapy is initiated. The results also demonstrate that peritoneal cavity tumors respond to the ganciclovir therapy upon activation with MC containing the TK gene.

With respect to Group 5 and 6 animals, the Group 6 animals survived longer than the Group 5 animals. The response of the murine mammary tumor (EMT 6.8) cells indicate that a different tumor type can be targeted by the Kbalb-STK cells. Moreover, the Kbalb-STK cells were effective in causing a bystander effect in this tumor.

These results demonstrate that the daughter cells (transduced cells) were able to migrate to the tumor in the intraperitoneal cavity. The transduced cells were injected into the subject animals after introduction of the parental cancer cells and migrated to or targeted the parental cells *in vivo*. Upon ganciclovir therapy, the transduced cancer cells which migrated caused cell death to the parental tumor cells *in situ* which resulted in prolonged survival of the animals.

EXAMPLE 3

DEMONSTRATION OF PRE-EXISTING TUMOR CELL KILLING *IN VIVO*

In this experiment, we undertook to form a variety of pre-existing tumors in the peritoneal cavity of mice. Upon formation of the tumors and the passage of one to five days, we then sought to effect tumor regression through therapy with Kbalb-STK cells and ganciclovir, also administered intraperitoneally.

In the experiment, six groups of mice were used to show the effect of Kbalb-STK cells on tumors that were derived from Kbalb-LNL cells previously injected into the peritoneum. Mice were injected i.p. as follows:

Group 1: 2×10^5 Kbalb-STK cells were injected on day 0 and given no further treatment as a control (n=6);

Group 2: 2×10^5 Kbalb-LNL cells were injected on day 0 and given no further treatment as a control (n=4);

Group 3 2×10^5 Kbalb-LNL cells were injected on day 0 and given 150 mg ganciclovir/kg i.p. b.i.d. for 2.5 days (total of 5 doses of ganciclovir) starting 5 days after injection (n=10);

Group 4: 2×10^5 Kbalb-LNL cells were injected on day 0 and then 1×10^6 Kbalb-STK cells were injected i.p. on day 5, followed by 150 mg ganciclovir/kg i.p. b.i.d. for 2.5 days starting on day 9 (n=8);

Group 5: 2×10^5 Kbalb-LNL cells were injected on day 0 and then 1×10^7 Kbalb-STK cells were injected i.p. on day 5, followed by 150 mg ganciclovir/kg i.p. b.i.d. for 2.5 days starting on day 9 (n=8); and

Group 6: 2×10^5 Kbalb-LNL cells were injected on day 0 and then 1×10^6 Kbalb-STK cells were injected i.p.on day 1, followed by 150 mg ganciclovir/kg i.p. .i.d. for 2.5 days starting on day 5 (n=12).

No survivors in Groups 1, 2 or 3 were observed by 21 days after injection of cells, consistent with the course of tumor progression without any effective treatment. Group 6 animals had a mean survival of 31.6 days giving a $p<0.01$ when compared to group 2 which had a mean survival of less than 19 days. Group 4 had a survival rate of 50% at 26 days and 25% at 43 days while Group 5 had a mean survival of 50% at 30 and 25% at 41 days after injection of Kbalb-LNL cells.

The results demonstrate that a preexisting tumor was driven to regression by administration of TK+ tumor cells and treatment with ganciclovir. Further, the results [illegible]

EXAMPLE 4

HOMING OF INTRAVENOUSLY INJECTED TUMOR CELLS TO TUMOR DEPOSITS PRESENT IN A SUBJECT

In further exploration of the ambits of migration or homing, we conducted the following experiment to determine if intravenous injection of the transduced tumor cells would target a pre-existing tumor.

Three Balb/c mice were injected with 1 x 10^6 Kbalb cells subcutaneously on day 0. On day 1, 3 and 5 the mice were injected in the tail vein with 1 x 10^6 Kbalb-STK tumor cells. Ten days later the subcutaneous tumor was isolated and seeded in culture. After five days in culture the cells were split into three culture plates and placed in medium containing either in G418, ganciclovir, or unsupplemented medium (DMEM with 10% calf serum) and ten days later colonies in the culture dish were counted. Too numerous to count (TNTC) was indicated as 100%.

The cells harvested from the tumor mass were selected in G418 and those cells were re-passaged in tumor bearing mice.

TABLE 3

Percent Colony Number After 10 Days In Culture

Passage	Animal	Unsupplemented	G418	Ganciclovir
1	1	100	NT	NT
	2	100	NT	NT
	3	100	2	100
2	1	100	20	5
	2	100	50	<1
	3	0	NT	NT

* NT = not tested

The results indicate that I.V. injection of HSV-TK gene-modified tumor cells into a subject (such as mice) with a tumor (such as subcutaneous tumors) leads to the migration of the modified tumor cells to the subcutaneous tumor. A significant number of gene-modified cells could be isolated from the tumor mass such that the isolated population could be killed by the addition of ganciclovir.

EXAMPLE 5

HOMING OF I.P. INJECTED CELLS TO RESIDENT TUMOR MASSES

In order to further determine the scope and degree of migration of transduced tumor cells to a resident tumor mass, the following experiment was conducted.

In the eperiment, tumors were generated in the peritoneum of mice by inoculating 200,000 Kbalb-LNL cells I.P. Nine days after the innoculation of the Kbalb-LNL tumors, 2 x 10^7 fluorescent labeled PA-1STK cells were injected I.P. into the mice. The PA-1STK cell line was ' ained by insertion of the STK vector into PA317 cells. The PA317 cells were obtained from Dr. Dusty Miller and is a murine fibroblast packaging cell line. PA-1STK cells produce the STK vector at a titer of about 2 x 10^5 virus vector particles/mL. The cells were fluorescently labeled through growing the PA-1STK cells with the compound DiI (Promega) according to the manufacturer's instructions. The cells could then be visualized by fluorescense microscpoy of tissue sections from treated animals.

One and three days after the injection of the PA-1STK cells, tissues were isolated and analyzed by fluorescent microscopy. The results from the fluorescence stuy are presented in Table 4.

TABLE 4

TISSUE	Day 1	Day 2
Tumor	+++	+++
Kidney	-	-
Spleen	-	-
Intestine	-	-
Liver	-	-

The results indicate that injected tumor cells efficiently migrate to a pre-existing resident tumor in a subject. Recovery of the TK+ cells from the subject that have migrated to a resident tumor would be accomplished by growing PA-1STK cells in medium containing ganciclovir (for example, as described in Example 4). Such cells are MC and could be subjected to additional rounds of migration/selection.

EXAMPLE 6

IN VIVO RESPONSE TO GANCICLOVIR THERAPY USING A HUMAN NEUROBLASTOMA CELL LINE: TUMOR INHIBITION ASSAY

The human neuroblastoma cell line, SK-N-MC (ATCC No. HTB 10) was transduced with the STK vector (as described in Example 1) and grown in medium containing G418 in order to generate the SKNMC-STK cell line. Three athymic nude BALB/c mice were injected with 1.5 x 10^6 SKNMC-STK cells s.q. and for two of the animals, ganciclovir therapy (150 mg ganciclovir/kg mouse i.p. daily injection) was initiated the following day (day 1). The remaining animal was not treated with ganciclovir. At day 6 tumor size was determined for each animal.

The untreated control animal had a tumor at the site of injection 6.5 mm in diameter. No tumor was detected in either of the two animals that received

ganciclovir therapy. This result demonstrated that TK vectors currently available adequately express the TK gene in human tumor cells *in vivo* so that ganciclovir therapy is effective. This experiment also demonstrated that human tumor cells are susceptible to killing by a subject *in vivo*.

The observation that human cells respond to this therapeutic method, shows that cancers from other species (i.e., human) are amenable to treatment by the disclosed method.

EXAMPLE 7

TK+ CELLS GENERATED *IN VIVO* BY TRANSFER OF THE TK GENE INTO A PRE-EXISTING TUMOR: TUMOR INHIBITION PROTOCOL USING RETROVIRAL VECTOR STOCK TO TRANSFER THE TK GENE INTO KBalb CELLS.

In order to determine if we could cause incorporation of the TK gene into a pre-existing TK- tumor, we ran the following experiment.

Balb/c mice were injected i.p. with 5 x 10^4 Kbalb tumor cells on day 0. Thereafter, on days 1, 2 and 3 the mice were injected i.p. with either the LNL or the STK viral vector stock (about 4.0 ml/injection; vector titer for either virus was about 1 x 10^6 cfu/ml) in DMEM with serum. Three groups of animals were followed:

Group 1: received LNL vector and ganciclovir therapy (150 mg/kg b.i.d.) for 2.5 days starting on day 5;

Group 2: received STK vector and no ganciclovir therapy; and

Group 3: received STK vector and ganciclovir therapy (150 mg/kg

There were no survivors in Group 2 by 18 days after injection of cells and no survivors after 26 days in Group 1. In Group 3, however, 25% of the mice were still alive at 35 days.

The results demonstrate that the STK vector was transferred into the resident tumor mass. Moreover, ganciclovir treatment resulted in prolonged survival after *in vivo* gene transfer into the tumor cells.

EXAMPLE 8

IN VIVO RESPONSE TO GANCICLOVIR THERAPY USING LETHALLY IRRADIATED TUMOR CELLS: TUMOR INHIBITION ASSAY *IN VIVO*

In order to determine the effectiveness of TK+ containing lethally irradiated cells, we ran the following experiment.

Two groups of 10 Balb/c mice were injected i.p. as follows:

Group 1: injected with 2 x 10^5 Kbalb-LNL cells on day 0 and then with 5 x 10^6 Kbalb-STK cells (lethally gamma irradiated with 3000 rads immediately prior to injection) i.p. on day 1 and again on day 2 and then given a course of ganciclovir therapy (150 mg/kg i.p. b.i.d. for 2.5 days) starting on day three; and

Group 2: injected with 2 x 10^5 Kbalb-LNL cells on day 0 and then with 5 x 10^6 Kbalb-LNL cells (lethally gamma irradiated with 3000 rads immediately prior to injection) i.p. on day 1 and again on day 2 and then given a course of ganciclovir therapy (150 mg/kg i.p. b.i.d. for 2.5 days) starting on day three.

The Group 2 controls that did not receive any Kbalb-STK cells had a survival rate of 20% at 20 days, 10% at 21 days and 0% at 24 days. The Group 1 animals had a survival rate of 100% at 25 days, 30% at 40 days, 10%

at 43 days and 0% at 68 days. Accordingly, the treatment of lethally irradiated daughter cells with ganciclovir *in vivo* led to the prolonged survival of animals carrying parental cells.

EXAMPLE 9

TISSUE CULTURE STUDY OF LETHALLY IRRADIATED Kbalb-STK AND Kbalb-LNL CELLS

As a follow-up to the experiment described in Example 6, and in order to determine the difference in effectiveness between "normal" transduced tumor cells and lethally irradiated transduced tumor cells, we conducted a tissue culture study.

In the study we looked at the toxicity of ganciclovir toward lethally irradiated Kbalb-STK and Kbalb-LNL cells. Cells were radiated at 3000 rads of gamma radiation, plated at subconfluence and then exposed to 10 μM ganciclovir at different times after plating. In no case were any colonies observed to grow after radiation. This finding shows that the 3000 rad dose was in fact lethal.

Further, Kbalb-LNL cells that were exposed to ganciclovir at 0, 2, 4 and 6 days after plating remained attached to the plates in small numbers at 28 days after plating. The cells were alive but unable to divide.

Kbalb-STK cells that were exposed to ganciclovir at 0 days after plating (i.e. ganciclovir added at the same time the cells were plated) were all killed by 7 days after plating. No cells were found that remained attached to the plate. Kbalb-STK cells exposed to ganciclovir at 2, 4 and 6 days after plating remained attached to the plates at 14 days after plating. However, by 28 days, none were observed to remain attached to the plates.

This result shows that lethally irradiated Kbalb-STK cells were sensitive to ganciclovir killing, particularly when exposed to ganciclovir immediately

DNA synthesis and repair after irradiation of cells, and particularly Kbalb-STK cells.

EXAMPLE 10

HISTOLOGIC EVIDENCE OF AN ANTI-TUMOR EFFECT FOLLOWING I.P. INJECTION OF GENE-MODIFIED CELLS INTO TUMOR BEARING MICE

The ability of HSV-TK tumor cells to generate a tumoricidal effect *in vivo* was analyzed histologically by observing the I.P. tumors after injection of the gene-modified tumor cells into tumor bearing hosts. Mice received an I.P. inoculum of 2 x 10^5 Kbalb-LNL cells on day 0. On day 6, the animals were injected I.P. with 1 x 10^7 PA-1STK cells and ganciclovir. Mice were sacrificed one day later and the peritoneal contents were examined.

We observed tumor necrosis histologically within 24 hours of inoculation of the gene-modified cells as compared to untreated tumor. The tumor necrosis originated within the center of the tumor mass which was 4-6 mm in diameter. These studies demonstrate that gene-modified cells can affect a tumor that is 4-6 mm in diameter which was embedded within the omentum. The quick onset (within 24 hours) of the necrosis and the hemorrhagic aspect which begins within the tumor mass indicates that the "bystander effect" is in part mediated by cytokines or chemokines which are released by either the gene-modified cells or nearby cells when the HSV-TK expressing cells are inoculated I.P. It is interesting to note that these murine KBalb-LNL tumors are equivalent in size to human ovarian tumors.

We can identified an increased number of immunological cells in the areas of hemorrhagic necrosis. Immunohistochemistry was performed to determine the cell type using antibodies to the following cells: CD3-T cells, MAC-1-macrophages, CD11-B cells. The results are shown in Table 5.

TABLE 5

	Cells per microscopic field	
	Mean	Standard Deviation
CD3	19.08	13.85
MAC-1	21.5	24.00
CD11	11.44	9.04

EXAMPLE 11

CYTOKINES RELEASED *IN VIVO* IN THE TUMOR IN RESPONSE TO

INJECTION OF HSV-TK GENE-MODIFIED CELLS

Since hemorrhagic tumor necrosis is histologically observed after treatment with gene-modified tumor cells, it is possible that soluble factors are released which mediate this effect. To determine if cytokines are released in response to gene-modified cells, we injected mice with 2 x 10^5 Kbalb-LNL cells intraperitoneally (I.P.) on day 0. On day 10, the mice were inoculated with PA-1-STK (2 x 10^7) cells and ganciclovir I.P. A control group was untreated or injected with PA-1-STK cells without GCV.

One, three, and five days later mice were sacrificed and tumors harvested. RNA was isolated from the tumor sample on the day of sacrifice. There were two mice in each group and the experiment was repeated once for a total of four mice per group. The samples were analyzed for cytokine m-RNA using PCR technology and the results of the samples from animals one and three days post treatment are shown in Table 6.

TABLE 6

Group	IL-1α	IL-10	TNF-α	IL-6	IFN-γ	MIP-1α	IL-2	IL-4	TGF-β	GMCSF
one day-con't	-	-	-	-	-	+	-	-	+-	
one day-exp	+	-	+	+	+	+	-	-	+-	
three day-con't	-	-	-	-	-	+	-	-	+-	
three day-exp	+	-	+	+	+	+			--	

+ four of four animals in each group expressed message

As will be observed, cytokine production was observed for at least the following: IL-1α, TNF-α, IL-6, IFN-γ, and GMCSF. The expression of each of these cytokines is strongly indicative of the role of cytokines in mediating the "bystander effect" in cancer therapy. Moreover, this experiment demonstrates that through use of the present invention, in addition to effecting a bystander effect cell death, cytokine production can be induced in a tumor which will assist in tumor therapy by normal immunological processes.

It is expected that TGF-β production was higher in the experimental animals than the controls. We plan to conduct quantitative PCR and look at expression dynamics in order to test the relative induction of cytokine production for each of the individual cytokines.

EXAMPLE 12

PRE-IMMUNIZED TUMOR BEARING ANIMALS TREATED WITH PA-1STK CELLS

Mice were pre-immunized with one million irradiated kbalb-LNL tumor cells. Six weeks later the mice were injected I.P. with one million unirradiated kbalb LNL cells. Approximately 10 days later the mice were injected with ten million irradiated PA-1STK cells and ganciclovir. Three days later the animals were sacrificed and m-RNA was harvested from the tumors and PCR was performed for the cytokines listed in Table 7, below.

TABLE 7

Group	IL-1α	IL-10	TNF-α	IL-6	IFN-γ	MIP-1α	IL-2	IL-4
three day-cont	+	-	+	+	-	+	-	-
three day-exp	+	-	+	+	+	+	-	-

TNF m-RNA production was also assayed for in the spleen, liver, and kidney. There was no difference between control and experimental animals, although both control and experimental animals showed TNF message in the spleen three days post treatment. Thus we can demonstrate the generation of

IL-1, IL-6, and TNF, and IFN m-RNA within the tumor in response to the injection of the HSV-TK positive tumor cells.

EXAMPLE 13

IDENTIFICATION OF CELLS WHICH ARE THE SOURCE OF TNF-α

Using immunohistochemistry we analyzed for TNF production in tumor samples three days after animals were injected with HSV-TK tumor cells by using an antibody to TNF. The antibody is described in Chensue *J Leukocyte Biol* 46:529 (1989). Through use of this technique, we can demonstrate that non-tumorous mononuclear cells stain for TNF within the tumor. There is a marked increase in the number of cells expressing TNF-α in the experimental group over the control group.

TABLE 8

Number of TNF staining cells/microscopic field

	Mean	Standard Deviation
Control	2.6	1.4
Experimental	18.1	18.6

EXAMPLE 14

POTENTIATION OF THE "BYSTANDER EFFECT" BY CYTOKINES

In this experiment we attempted to demonstrate that a cytokine (i.e., IL-1) could be used to enhance the bystander effect in a subcutaneous tumor model. To this end, 1 x 10^6 Kbalb tumor cells were injected subcutaneously into mice. The injected Kbalb tumor cells consisted of varying mixtures of Kbalb LNL, Kbalb STK, and Kbalb II[illegible] cells day

The mice were then treated with ganciclovir for between about 2.5 and 5 days, twice per day, using 150 mg/Kg mouse ganciclovir, beginning when tumor diameter was 2.5 mm. Tumor regression was assayed as shown below in Table 9.

TABLE 9

GROUP	% LNL	% HSV-TK	% IL-1	% Regression
1	100	0	0	0
2	0	100	0	100
3	0	0	100	0
4	47.5	5	47.5	100
5	50	0	50	0
6	95	5	0	0
7	0	5	95	0

Thus, the IL-1 cytokine was shown to potentiate the bystander effect.

EXAMPLE 15

PANCREATIC CANCER PROTOCOL

As an extension of the above-decribed therapeutic procedures, a pancreatic cancer protocol is in progress. In the protocol, the PA-1STK cell line (Example 5) and the autologous cells are used. The patient is prevaccinated with the cell mixture containing approximately 5 x 10^6 PA-1STK cells and 5 x 10^6 autologous pancreatic cancer cells from the patient. Prevaccination will be accomplished subcutaneously, for example in the arm.

Approximately 6 to 8 weeks later, patient will be treated in dose escalation protocols as described in WO 93/02556, Example 16.

Regression of the pancreatic cancer in the subject will be followed. We expect that the prevaccination with the mixed cell population will create a

specific target tumor for the PA-1STK cells. Upon their administration, they will efficiently and specifically migrate to the tumor and, with gavciclovir therapy, lead to tumor regression.

INCORPORATION BY REFERENCE

To the extent that any reference cited herein has not been expressly incorporated by reference, any reference cited is hereby expressly incorporated by reference in its entirety. Moreover, with respect to any review article cited herein, it is intended that both the review article and its references are incorporated by reference herein in their entirety.

EQUIVALENTS

It is to be understood that the preceding examples may be varied within the scope and spirit of the present invention with regard to the disease states that may be ameliorated and with regard to the methods of gene transfer and gene vectors used. Because many embodiments of the present invention are possible within the scope thereof, it is to be construed that the present invention is not limited to the specific embodiments thereof, which are exemplary. Accordingly, the scope of the invention should be construed in light of the appended claims and any equivalents thereof.

WHAT WE CLAIM IS:

1. A method to generate a migration selected cell population that will migrate from a first site of introduction to a subject to a specific cell or tissue-type at a second site in the subject, comprising:

providing a first cell population that exhibits associational tendencies with the cell or tissue-type at the second site;

introducing the first cell population into the subject at a first site;

allowing a portion of the first cell population to migrate to and associate with the cell or tissue-type at the second site; and

recovering the portion of the first cell population from the second site that migrated thereto from the first site as the migration selected cell population.

2. The method of Claim 1, wherein the associational tendencies are selected from the group consisting of aggregation with the cell or tissue type *in vitro*, migration to the cell or tissue-type *in vitro*, homing to the cell or tissue-type *in vitro*, migration to the cell or tissue-type *in vivo*, and homing to the cell or tissue-type *in vivo*.

3. The method of Claim 1, wherein, in the introducing step, the mode of introduction is selected from the group consisting of injection, implantation, and application.

4. The method of Claim 1, wherein the cell population comprises cancer cells and the cell or tissue type at the second site comprises cancerous cells.

5. The method of Claim 4, wherein the cancer cells are genetically altered to include a gene selected from the group consisting of a susceptibility enhancing gene, a cytokine gene, a lymphokine gene, a histocompatibility antigen gene, a T cell receptor gene, a chimeric cytokine gene, an adhesion molecule gene, a tumor antigen gene, and a virus protein gene.

6. The method of Claim 5, wherein the gene is a human or murine gene that is selected from the group consisting of a susceptibility enhancing gene, a cytokine gene, and a lymphokine gene.

7. The method of Claim 4, wherein the cancer cell is selected from the group consisting of ovarian carcinomas, breast carcinomas, leptomeningeal carcinomatosis, colon carcinoma, glioblastoma, a B cell lymphoma, a T cell lymphoma, a B cell leukemia, a T cell leukemia, neuroblastoma, soft tissue sarcoma, cervical carcinoma, lung carcinoma, urinary carcinoma, bladder carcinoma, stomach carcinoma, adrenal cortex carcinoma, endometrial carcinoma, prostate carcinoma, fibrosarcoma, adenocarcinoma, prostate carcinoma, liver carcinoma, pancreatic carcinoma, and biliary duct carcinoma.

8. A method to deliver a transduced tumor cell to a tumor in a subject, comprising:

providing a transduced tumor cell that, prior to transduction, is similar in type to the tumor in the subject, and, wherein upon transduction, the transduced tumor cell comprises a gene that is not present in significant quantities in the tumor;

introducing the transduced tumor cell into the subject; and

allowing the transduced tumor cell to migrate to the tumor.

9. The method of Claim 8, wherein the tumor cell is transduced with a gene that renders it more susceptible to a therapeutic agent.

10. The method of Claim 9, wherein the gene is a thymidine kinase gene and therapeutic agent is a nucleoside analogue.

11. The method of Claim 10, wherein the thymidine kinase gene is derived from a herpes virus.

12. The method of Claim 11, wherein the nucleoside analogue is selected from the group selected from acyclovir and ganciclovir.

13. A migration selected cell line, comprising:

a cell population derived from cells removed from a first cell or tissue type in a subject following migration of the cells from a second site in the subject.

14. The cell line of Claim 13, wherein the cell population comprises cancer cells and the second site of the subject comprises a tumor.

15. A method for treating cancer in a first subject having a site of cancerous tissue, comprising:

removing a population of cancerous cells from the first subject;

transducing the cancer cells with a gene that renders the cells more susceptible to a therapeutic agent;

introducing the transduced cancer cells into the first subject and allowing the transduced cells to migrate to the site of cancerous tissue; and

treating the subject with the therapeutic agent, wherein, upon death of the transduced cancer cells a significant number of cancerous cells in the cancerous tissue of the subject will be killed.

16. The method of Claim 15, wherein the gene is a thymidine kinase gene and therapeutic agent is a nucleoside analogue.

17. The method of Claim 16, wherein the thymidine kinase gene is derived from a herpes virus.

18. The method of Claim 16, wherein the nucleoside analogue is selected from the group selected from acyclovir and ganciclovir.

19. The method of Claim 15, further comprising, prior to the introducing step, the steps of:

introducing the transduced cancer cells into a second subject at a first site, the second subject having cencerous cells at a second site of a similar type to the cancerous cells in the first subject;

allowing the transduced cancer cells to migrate to the second site in the second subject; and

removing the transduced cancer cells from the second site of the second subject.

20. The method of Claim 19, wherein the second subject is allogenic or xenogenic with the first subject.

21. The method of Claim 19, wherein the second subject is genetically similar to the first subject.

22. The method of Claim 20, wherein the second subject is a mouse and the first subject is a human.

23. The method of Claim 15, further comprising, prior to the transducing step, the steps of:

introducing the transduced cancer cells into a second subject at a first site, the second subject having cencerous cells at a second site of a similar type to the cancerous cells in the first subject;

allowing the transduced cancer cells to migrate to the second site in the second subject; and

removing the transduced cancer cells from the second site of the second subject.

24. The method of Claim 19, wherein the second subject is allogenic or xenogenic with the first subject.

25. The method of Claim 19, wherein the second subject is genetically similar to the first subject.

26. The method of Claim 24, wherein the second subject is a mouse and the first subject is a human.

27. A method to induce cytokine production in a subject having a tumor, comprising:

providing a population of modified cancer cells that are similar in type to cells in the tumor in the subject, the cancer cells including a

introducing the modified cancer cells into the subject in a manner designed to bring the cancer cells into proximity with the tumor; and

administering the agent to the subject in order to kill the modified cancer cells, wherein, upon the death of the modified cancer cells, cytokine production is induced in the tumor.

28. The method of Claim 27, wherein the gene is a thymidine kinase gene and therapeutic agent is a nucleoside analogue.

29. The method of Claim 28, wherein the thymidine kinase gene is derived from a herpes virus.

30. The method of Claim 28, wherein the nucleoside analogue is selected from the group selected from acyclovir and ganciclovir.

31. The method of Claim 27, wherein the cytokine production that is induced is selected from the group consisting of IL-1α, IL-6, TNF-α, IFN-γ, and GMCSF.

32. A fused cell cell line, comprising:

a cell line derived from T helper 2 cells fused with a tumor cell line from a host animal.

33. A fused cell cell line, comprising:

a cell line derived from CTCL cells fused with a tumor cell line from a host animal.